REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	18 Dec 97	

4. TITLE AND SUBTITLE
REGULATION OF GnRH RECEPTOR mRNA: INTERACTION OF GnRH AND ESTRADIOL

5. FUNDING NUMBERS

6. AUTHOR(S)
Tara Elizabeth Nolan

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Colorado state University

8. PERFORMING ORGANIZATION REPORT NUMBER
97-150

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
THE DEPARTMENT OF THE AIR FORCE
AFIT/CIA, BLDG 125
2950 P STREET
WPAFB OH 45433

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION AVAILABILITY STATEMENT
Unlimited Distribution
In Acdordance With AFI 35-205/AFIT Sup 1

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

14. SUBJECT TERMS	15. NUMBER OF PAGES
	53
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT

THESIS

REGULATION OF GnRH RECEPTOR mRNA: INTERACTION OF GnRH AND ESTRADIOL

Submitted by
Tara Elizabeth Nolan
Cell and Molecular Biology

In partial fulfillment of the requirements
for the Degree of Master of Science

Colorado State University
Fort Collins, Colorado
Spring 1998

COLORADO STATE UNIVERSITY

November 24, 1997

We hereby recommend that the thesis prepared under our supervision by Tara E. Nolan entitled "Regulation of GnRH Receptor mRNA: Interaction of GnRH and Estradiol" be accepted as fulfilling in part requirements for the degree of Master of Science.

Committee on Graduate Work

Michael K. Fox

Jerry M. Nett

Colin M. Clay

Advisor

Michael K. Fox

Department Head/Director

ABSTRACT OF THESIS

REGULATION OF GnRH RECEPTOR mRNA: INTERACTION OF GnRH AND ESTRADIOL

The integrated functions of the hypothalamus, anterior pituitary gland, and ovaries serve to regulate normal female reproductive function. The hypothalamus secretes Gonadotropin-Releasing Hormone (GnRH), a peptide hormone, in a pulsatile manner. GnRH stimulates the anterior pituitary gland via binding to specific, high-affinity receptors on the plasma membrane of gonadotrophs. GnRH binding initiates a cascade of intracellular events resulting in synthesis and secretion of the gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH), and also synthesis and insertion of its own receptors into the plasma membrane. When gonadotropins are released into circulation, they exert their primary biological effects at the gonads. Ovarian hormones, progesterone, inhibin, and estradiol, feedback effects at both the hypothalamus and pituitary gland. Progesterone reduces the pulse frequency and amplitude of GnRH being released from the hypothalamus, thus, decreasing gonadotropin secretion. It is important to note that estradiol does not stimulate secretion of gonadotropins into the circulation. Inhibin is a direct regulator of FSH synthesis and secretion.

A high concentration of GnRH and estradiol, as seen prior to the pre-ovulatory surge, results in an increase in production of gonadotropins and GnRH receptor. The rise in GnRH receptor numbers increases gonadotroph sensitivity

to GnRH and thus the amounts of LH and FSH secreted by the cell in response to GnRH. The mode of GnRH reaching the pituitary gland is, however, critical. In a pulsatile manner GnRH stimulates an increase in GnRH receptors, whereas GnRH delivered via a continuous infusion results in desensitization of gonadotrophs which is characterized by a decrease in the concentration of GnRH receptors. As mentioned above, estradiol increases the numbers of GnRH receptors, presumably through an increase in transcription rates of the GnRH receptor gene. Given that estradiol and GnRH are often combined in therapeutic regimes we asked the question, if estradiol is able to increase GnRH receptor synthesis, will a continuous infusion of GnRH cause GnRH receptor to internalize faster than estradiol can stimulate synthesis?

To address these questions, ovariectomized ewes were administered GnRH continuously via subcutaneous Alzet osmotic mini-pumps for 136 hours, to desensitize the anterior pituitary gland. Twelve hours prior to the end of treatment, half of the ewes were given a bolus intramuscular injection of estradiol. Jugular blood samples were collected for the duration of the experiment and pituitary glands were collected at the termination of the experiment.

The continuous infusion of GnRH caused desensitization of the gonadotrophs. Treatment with estradiol caused concentrations of serum LH to increase in both the saline-treated ewes and in ewes pre-treated with a continuous infusion of GnRH. However, it is important to note the onset of this increase was 4 ½ hours earlier in ewes treated with GnRH. We presume this is

because high levels of circulating GnRH from the pumps was readily available as new GnRH receptors were inserted in to the plasma membrane.

Continuous administration of GnRH caused a decrease in the steady-state levels of mRNA encoding GnRH receptor and number of GnRH receptors. In contrast, treatment with estradiol induced an increase in levels of mRNA for GnRH receptor and number of GnRH receptors relative to controls and in the group receiving continuous GnRH. These results support the conclusion that estradiol, acting on the pituitary gland, stimulates transcription of the GnRH receptor gene. Moreover, desensitization of gonadotrophs by continuous infusion of GnRH and the resulting decrease in mRNA for GnRH receptor and concentrations of GnRH receptors can be overridden by exogenous estradiol. These results support the hypothesis that estradiol may override inhibition of GnRH receptor caused by continuous exposure of gonadotrophs to GnRH, and suggest that new GnRH receptors are synthesized and inserted into the plasma membrane within 6 hours after administration of estradiol.

Tara E. Nolan
Cell and Molecular Biology
Colorado State University
Fort Collins, CO 80523
Spring 1998

TABLE OF CONTENTS

ACKNOWLEDGEMENTS

I express my deepest gratitude to Dr. Adele Turzillo for her guidance and advice, for wrestling sheep with me, being a patient teacher, and for not making me feel stupid when I had difficulty with dilution calculations—each time.

I feel this thesis was a team effort and I would like to thank everyone from the lab who helped me draw blood samples—Keith Rollyson, David Howarth, Matt Allen, Adele Turzillo, Jenny Juengel, Rick Silva, Eric McIntush, Michael Royals, and Mike Gallegos. I now have a great deal of respect for the amount of time and teamwork that is behind each paper published.

I would like to thank Tina Garner and Xiaoming Sha for helping me through endless assays and repairing various pipetters which I developed a knack for breaking.

I owe special thanks to my brothers—Michael and Jimmy.

For aid in labeling tubes, I would like to thank: Michael, Jimmy, Daniel and Loel—pizza and movies do not express my gratitude. Also, Loel was my editor extraordinaire

Dr. Colin Clay, thank you for standing by me. Your support carried me through the rough spots so I could enjoy the fruits of accomplishment

Dr. Terry Nett, thank you for sharing your sheep wrangling expertise and enabling me to successfully complete my master's degree in such a short time period, in spite of my talking like a "valley girl".

Special thanks goes to Dr. John Obringer, Lt. Colonel, ret. For his faith in my ability and for pushing me into areas I would not go on my own.

CHAPTER ONE

REVIEW OF LITERATURE

A. Hypothalamic-Pituitary-Gonadal Axis

The integrated functions of the hypothalamus, anterior pituitary gland, and ovaries serve to regulate normal female reproductive function. Figure 1 illustrates this integration as a feedback loop (Pierce and Parsons, 1981) termed the "hypothalamic-pituitary-gonadal axis." In particular, the neurosecretory cells located in the preoptic area of the hypothalamus produce Gonadotropin-Releasing Hormone (GnRH) (Wheaton et al., 1978; Braden and Conn, 1991), a peptide hormone that is secreted into the hypophyseal portal circulation (Green and Harris, 1947) for transport to the anterior pituitary gland (Amoss et al., 1971). GnRH binds to receptors on the plasma membrane (Marion and Conn, 1983) of gonadotrophs, thus stimulating the synthesis and secretion of the gonadotropins (Arimura and Schally, 1970; Schally et al., 1972; Clayton and Catt, 1981; Pierce and Parsons, 1981), follicle-stimulating hormone (FSH) and luteinizing hormone (LH), as well as synthesis of GnRH receptor (GnRH receptor).

In the female, as reviewed by Gharib et al. (1990), when gonadotropins are released into the circulation, they exert their primary biological effects at the

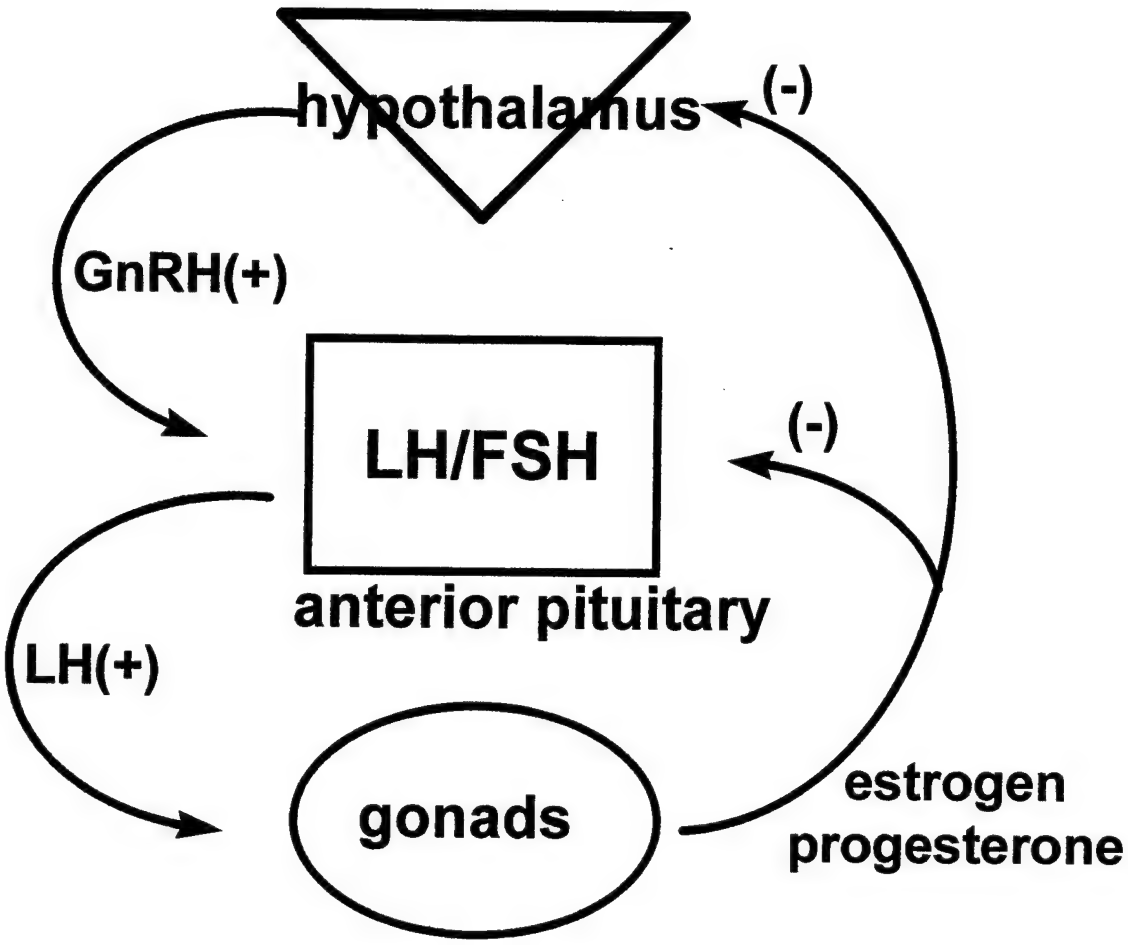

Figure 1. Hypothalamic-pituitary-gonadal axis. GnRH from the hypothalamus stimulates LH and FSH secretion from the anterior pituitary gland. LH and FSH stimulate gametogenesis and steroidogenesis at the level of the gonads. The gonadal steroid hormones, estrogen and progesterone, then typically act in a negative feedback fashion to suppress further production of the gonadotropins

ovary, regulating steroidogenesis and gametogenesis (Gharib et al., 1990). Follicle-stimulating hormone regulates early stages of follicular development (Gharib et al., 1990) whereas, LH regulates later stages of follicular development as well as induces the luteal phase of the estrous cycle by stimulating ovulation and formation of the corpus luteum (Gharib et al., 1990).

The feedback effects of gonadal hormones to regulate hypothalamic and anterior pituitary gland function (Pierce and Parsons, 1981; Karsch et al., 1987; Gharib et al., 1990; Kaiser et al., 1997) is evident in the regulation of GnRH receptor and the gonadotropins themselves. Three hormones are of interest to this regulation; progesterone, inhibin, and estradiol. The latter hormone has the ability to transverse the gonadotroph plasma membrane, bind to an intracellular receptor, and stimulate the synthesis of GnRH receptor. Thus, an increased pulse frequency of GnRH and a high concentration of estradiol result in an increased production of gonadotropins and GnRH receptor.

B. Regulation of Pituitary Function

I. Hypothalamus

The hypothalamus lies at the basal part of the diencephalon below the thalamus, forming the walls and lower part of the third ventricle of the brain (Reichlin, 1967). Within the hypothalamus are clusters of neurons that are symmetrically located around the third ventricle (Reichlin, 1967; Jennes et al., 1988). The endocrine hypothalamus consists of neurons which secrete the neurohormones that regulate anterior pituitary function (Reichlin, 1967). Synaptic

contacts from other neuronal elements connect the endocrine hypothalamus to the rest of the central nervous system (Jennes et al., 1988). Information flow from other brain centers is relayed to hypothalamic neurons which then secrete their neurohormones into the pituitary portal vasculature of the median eminence. Hypothalamic hormones stimulate the endocrine cells in the pituitary to release their specific hormones (Reichlin, 1989; Schally, 1978). Of particular interest in regulation of reproductive function is the hypothalamic hormone, GnRH.

First isolated and characterized as a hypothalamic releasing factor in 1971 (Matsuo et al., 1971), GnRH is a decapeptide hormone (pyroGlu^{1}-His^{2}-Trp^{3}-Ser^{4}-Tyr^{5}-Gly^{6}-Leu^{7}-Arg^{8}-Pro^{9}-Gly^{10}amide)(Schally et al., 1971; Burgus et al., 1971) synthesized mainly in the arcuate nucleus (Wheaton, 1978; Braden and Conn, 1991) of the hypothalamus. GnRH is transported from the hypothalamus to the median eminence where it is released in a pulsatile manner (Carmel et al., 1975) into the portal vasculature (Kochman et. Al., 1975; Swift and Crighton, 1979; Clayton and Catt, 1981) with a frequency of approximately one pulse per hour (Knobil et al., 1980) during the follicular phase. Secretion of GnRH increases during the follicular phase of the ovine estrous cycle (Clarke et al., 1987; Moenter et al., 1991).

The binding of GnRH to receptors on gonadotrophs is the first and vital step in initiating the cascade of intracellular events (Arimura and Schally, 1970; Schulling et al., 1987; Braden and Conn, 1991) necessary for normal reproductive function. Without this initiation step, regulation of the reproductive

cycle is lost. Perhaps the best example of this is a naturally-occurring strain of mice that harbor a mutation in the GnRH gene that prevents correct synthesis of GnRH (Mason et al., 1986). Normal GnRH stimulates the secretion of LH and FSH as illustrated in figure 2, however, these hypogonadal mice produce non-functional GnRH. Therefore, they neither synthesize nor secrete the gonadotropic hormones resulting in complete lack of normal gonadal function (Aschner, 1912; Brolin, 1945; Westman and Jacobson, 1940).

II. Anterior Pituitary Gland

The hypophysis, or anterior pituitary gland, is located directly below the hypothalamus in the sella turcica of the sphenoid bone (Rhodin, 1974; Reichlin, 1989). The pituitary gland is comprised of two separate lobes: the anterior pituitary gland (adenohypophysis) and the posterior pituitary gland (neurohypophysis).

The anterior pituitary gland is made up of at least five endocrine cell types: somatotrophs, lactotrophs, thyrotrophs, corticotrophs and gonadotrophs.. Each synthesizes and secrets specific hormones which regulate biological processes. Somatotrophs and lactotrophs comprise 70% of the anterior pituitary cells (Reichlin, 1989); somatotrophs synthesize and secrete growth hormone and lactotrophs secrete prolactin (Reichlin, 1989). Thyrotrophs, the largest of the cell types, secrete thyroid-stimulating hormone (Reichlin, 1989). Corticotrophs comprise approximately 10% of the cell types and stimulate the adrenal cortex resulting in glucorticoid secretion (Reichlin, 1989). The

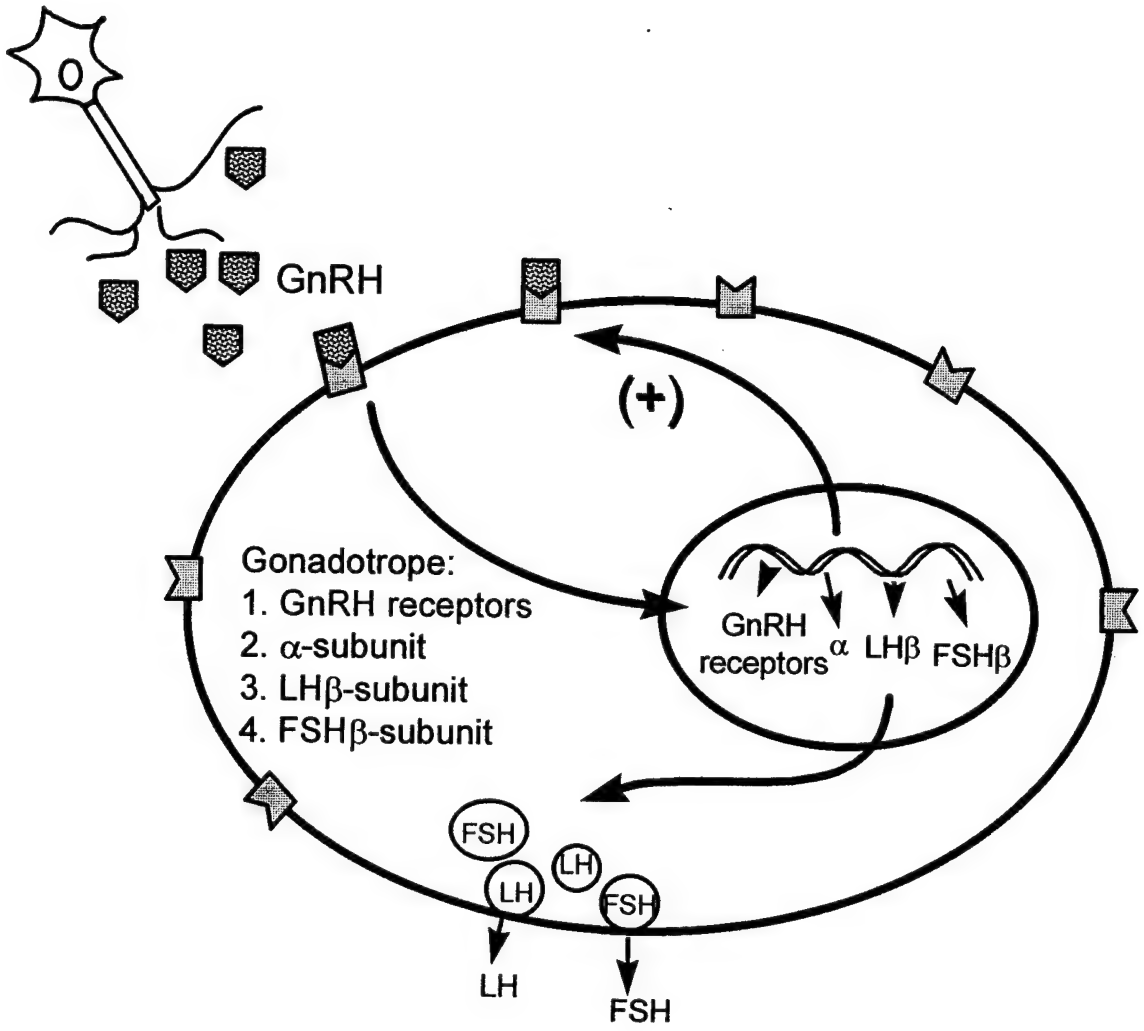

Figure 2. Hypothalamic GnRH binds to specific, high-affinity GnRH receptors located on gonadotrope cells and stimulates expression of the genes encoding the GnRH receptor, the common α-subunit, the LHβ-subunit, and the FSHβ-subunit. Additionally, GnRH induces the release of the LH and FSH stored in secretory granules.

remaining 8-12% of the cells are gonadotrophs (Ibrahim et al., 1986) which synthesize and secrets the gonadotropins.

a. Gonadotrophs

Gonadotrophs synthesize and secreteLH and FSH (gonadotropins) in response to GnRH. That GnRH exerts its biological effects via a membrane receptor has been long established (Marian and Conn, 1983), however, not until 1992 when cDNAs encoding the GnRH receptor were first reported was there any information as to the structure of this receptor (Reinhart et. al., 1992; Tsutsumi et. al., 1992). The cDNA for murine GnRH receptor encodes a 327 amino acid protein with 7 membrane-spanning domains (Reinhart et. al., 1992; Tsutsumi et. al., 1992) which is consistent with membership of this receptor in the superfamily of G-protein coupled receptors (Probst et al., 1992). The GnRH receptor protein structure appears to be conserved across species (Kaiser et. al., 1997). Binding of GnRH to the gonadotroph plasma membrane receptor triggers an intracellular cascade resulting in the synthesis of α-subunit, LHβ and FSHβ-subunits, and GnRH receptor (Marian and Conn, 1983).

The gonadotroph is also regulated by the steroid hormone estradiol which acts via an intracellular receptor (Jensen and Jacobson, 1962; O'Malley et al., 1969; Evans, 1988; Tsai and O'Malley, 1994). Ligand-receptor binding stimulates the gonadotroph to transcribe and translate mRNA for GnRH receptor and to insert GnRH receptors into the plasma membrane. Although some effects of

GnRH and estradiol on gonadotroph functions are similar, these hormones presumably work through functionally different mechanisms. As discussed above GnRH exerts its actions through a G-protein coupled receptor (Marion and Conn, 1983) whereas estradiol acts via an intracellular receptor (Jensen and Jacobson, 1962; O'Malley et al., 1969; Evans, 1988; Tsai and O'Malley, 1994) as illustrated in figure 3.

b. Gonadotropins

As members of the glycoprotein hormone family (Pierce and Parsons, 1981; Jameson and Hollenberg, 1993), LH and FSH are comprised of a specific β-subunit, that is coupled with an α-subunit common to LH, FSH, and TSH (Pierce and Parsons, 1981). Carbohydrates comprise 15 to 30% of the molecular weight of the gonadotropins (Baenziger, 1990). The α-subunit has two asparagine (N)-linked oligosaccharides. The LHβ has one and FSHβ has two N-linked oligosaccharides (Pierce and Parsons, 1981; Baenziger, 1990).

The α- and β-subunits are each encoded by a single copy gene (Kim et. al., 1988; Gharib et. al., 1989; Watkins et al., 1987; Jameson et. al., 1988) and was initially isolated from humans (Fiddes and Goodman, 1981). In some neoplasms, the α-subunit is produced in the absence of β-subunits (Vaitukaitis, 1979), indicating that there is less stringent control of α-gene expression than β-gene expression (Jameson et al., 1989). In support of this concept, using the OVX-HPD model, Di Gregorio and Nett (1995) showed steady-state levels of

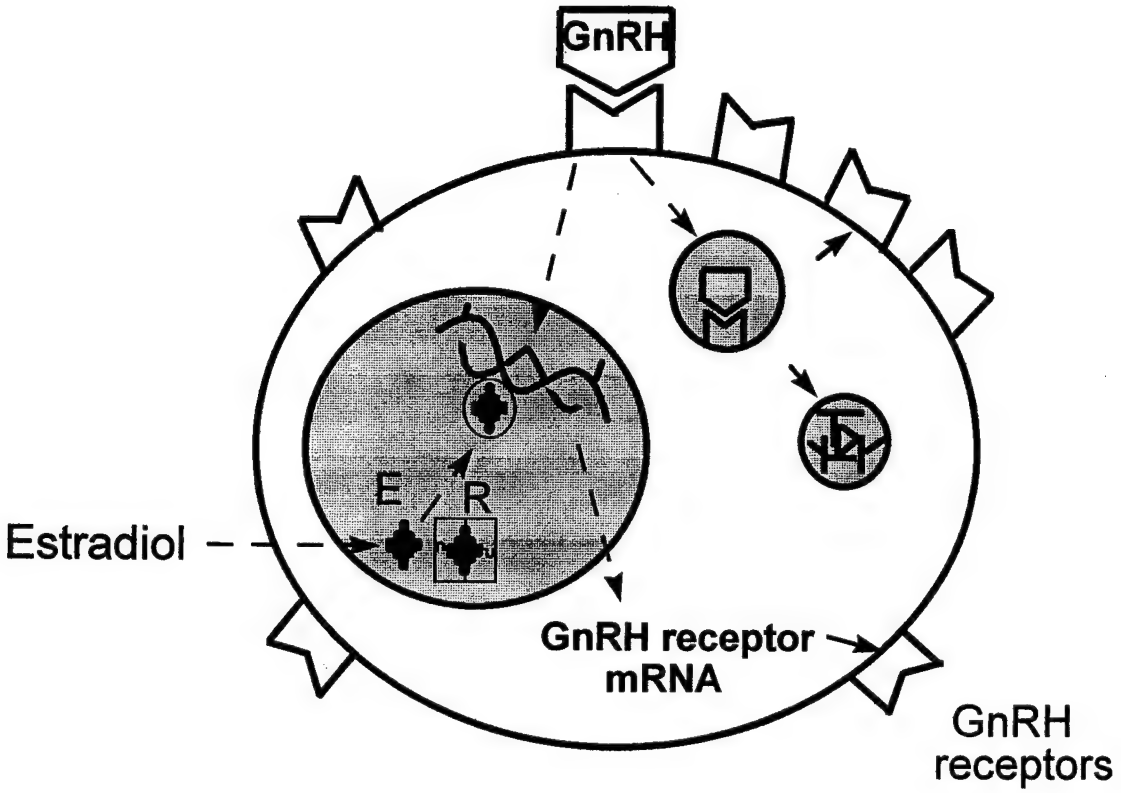

Figure 3. Estradiol is a steroid receptor which binds to an intracellular receptor, inducing a conformational change increasing receptor affinity for the DNA. Estradiol stimulates gene expression of α-subunit, LHβ-subunit, FSHβ-subunit and GnRH receptor via a mechanism independent of GnRH.

mRNA for α-subunit were not directly regulated by estradiol at the level of the pituitary gland. However, the β-subunits of the gonadotropins appear to be directly negatively regulated by estradiol (Alexander and Miller, 1982; Phillips et al., 1988; Sakurai et al., 1996; Herring et al., 1991; Nett et al., 1991). This indicates that the β-subunit not only lends specificity to the gonadotropins but is a rate-limiting step in LH and FSH protein synthesis.

c. GnRH regulation of pituitary function

GnRH binding to its receptor in the pituitary gland causes release of LH and FSH into the circulation. Thus, the effects of GnRH on gonadotrophs can be studied by monitoring LH secretion (Miller et al., 1990). The sensitivity of the pituitary gland depends on the concentration of GnRH receptors (Miller et al., 1990). The amount of GnRH secreted by the hypothalamus will affect pituitary responsiveness because GnRH is a homologous regulator of it's own receptor (Nett et al., 1981).

1. GnRH receptor

Concentrations of plasma membrane GnRH receptor (Clarke et al., 1983; Hamernik et al., 1986; Esbenshade et al., 1986; Culler and Negro-Vilar, 1986; Clarke et al., 1987; Hamernik and Nett, 1988; Gregg and Nett, 1989) and mRNAs for gonadotropin subunits maintained by GnRH (Hamernik and Nett, 1988) are proportional to the frequency and amplitude of the GnRH pulses

(Papavasiliou et al., 1986). In response to GnRH stimulus, GnRH receptors are inserted or removed from the plasma membrane of gonadotrophs (Nett et al., 1984). The sensitivity of the pituitary gland is based on the concentration of GnRH receptor such that more receptors means the pituitary gland can have a greater response to a GnRH challenge. Estradiol stimulates an increase in frequency and amplitude of GnRH pulses during the follicular phase of the estrous cycle. Conversely, during the luteal phase, high levels of progesterone negatively regulate the hypothalamus and pituitary gland, decreasing the frequency of GnRH pulses which, in turn, leads to a decrease in the concentration of GnRH receptor. In the absence of ovarian steroids, variations in GnRH pulse frequency does not increase the concentration of GnRH receptor (Clarke et al., 1987b). This indicates that, in an intact animal, estradiol enhances the increase in GnRH receptor prior to the pre-ovulatory surge.

Regulation of GnRH receptor numbers is complicated and reflects changes in rates of internalization, recycling, degradation and synthesis. Sternberger and Petrali (1975) reported the presence of GnRH receptor on secretory granule membranes. When secretory granules fuse with the plasma membrane to release LH, GnRH receptor numbers increase (Duello and Nett, 1980; Nett et al., 1981). However, this increase is not sufficient to explain the number of membrane receptors seen during the pre-ovulatory surge.

Also it has been shown that GnRH receptor can be incorporated into the plasma membrane independent of LH secretion (Gregg and Nett, 1989). Thus other mechanisms besides fusion of secretory granules with the plasma

membrane must be in place to increase numbers of GnRH receptors. Typically an increase in the number of GnRH receptors is coupled with an increase in serum levels of LH as one effect of GnRH binding is to stimulate secretion LH. In fact, since the isolation of the cDNA encoding GnRH receptor, several labs have demonstrated that increases in GnRH receptor is associated with increased GnRH receptor mRNA. These data indicate regulation of GnRH receptor concentration occurs at the transcriptional level (Sakurai et al., 1992; Sakurai et al., 1997).

Regardless of the precise mechanism, it is clear that GnRH is an important regulator of its own receptor (Nett et al., 1981), however, the mode by which the pituitary gland is exposed to GnRH is critical. Depriving the pituitary gland of GnRH by hypothalamic-pituitary-disconnection results in decreased numbers of GnRH receptors (Clarke et al., 1987a; Gregg and Nett, 1989). Turzillo et al. (1995a) demonstrated that normal GnRH receptor numbers can be restored through exogenous pulsatile GnRH replacement. Therefore, maintenance of steady-state concentrations of GnRH receptor and mRNA requires consistent GnRH stimulation (Sakurai, 1997, 189).

An experimental model in which the anterior pituitary gland is continuously exposed to GnRH was developed to determine the significance of pulsatile release of GnRH from the hypothalamus. Previously, GnRH has been shown to be a homologous regulator of it's own receptor in cycling ewes (Tsutsumi, 1993, 1625; Nett et al., 1981), in anestrous ewes (Khalid et al., 1988) and OVX-HPD ewes (Hamernik and Nett, 1988). It has also been shown that an increase in

GnRH receptor concentration in response to GnRH stimulus may contribute to the pre-ovulatory LH surge (Crowder et al., 1984). However, if OVX ewes are administered a continuous infusion of GnRH, the anterior pituitary gland becomes refractory to GnRH (Nett et al., 1981; Crowder et. al., 1986). Although the exact reduction in numbers of GnRH receptors to cause desensitization of the gonadotrophs is unknown, Wise et al. (1984) demonstrated that desensitization does occur with a 50% reduction in GnRH receptor concentration. Thus, GnRH receptor concentration may influence tonic LH release, but maximum LH release is not affected unless receptor concentration drops below 50% (Wise et. al., 1984). Desensitization is quickly reversible once continuous GnRH input is discontinued (Crowder et al., 1986). To summarize effects: pulsatile GnRH maintains or restores normal GnRH receptor levels. An absence of GnRH decreases concentrations of GnRH receptor. Likewise, continuous GnRH decreases numbers of GnRH receptor.

2. mRNA for LHβ, FSHβ and α-subunit

GnRH stimulates the synthesis of mRNA for LHβ, FSHβ, α-subunit and GnRH receptor. Removal of GnRH stimulus through passive immunoneutralization (Sakurai et al.,1997) of sheep or hypothalamic-pituitary disconnection (Hamernik et al., 1986; Nett, 1990) leads to a decrease in mRNA for LHβ, FSHβ, and α-subunits and GnRH receptor. As expected exogenous pulsatile GnRH increases concentrations of mRNA encoding ovine gonadotropin

subunits and GnRH receptor (Hamernik and Nett, 1988; Turzillo, 1995) highlighting the correlation between GnRH-stimulated synthesis and secretion of gonadotropins and synthesis of and insertion of GnRH receptor into the plasma membrane (Brooks, 1994). The activity of both the human and bovine α-subunit promoters are stimulated by GnRH (Hamernik et al., 1992) and α-subunit mRNA is induced by GnRH in a dose and time-dependent manner (Windle et al., 1990). There is evidence that the gonadotropin subunit genes do not respond equally to pulsatile GnRH stimulation (Marshall et al., 1991; Kile and Nett, 1994). LHβ gene expression increases with rapid GnRH pulses (Papavasiliou et al., 1986) while FSHβ gene expression increases in response to slow pulses (Dalkin et al., 1989). The ubquitious α-subunit does not have a characterized response (Dalkin et al., 1989; Weiss et al., 1990).

3. LH and FSH secretion

Two important determinants of the amount of LH and FSH secreted, as reviewed by Braden and Conn (1991), into the peripheral circulation are the amount of GnRH that reaches the pituitary gland (Caraty et al., 1982; Clarke et al., 1987a) and the concentration of GnRH receptor on the plasma membrane of the pituitary gland (Wise et al., 1984; Crowder et al., 1986; Brooks et al., 1993). Gonadotropins are released into circulation in different amounts at different times during the estrous cycle (Clayton and Catt, 1981). Accordingly, the sensitivity,

i.e. concentration of GnRH receptor in the pituitary gland, is an important determinant of the magnitude of the secretory response (Laws et al., 1990).

GnRH stimulates LH secretion (Hamernik and Nett, 1988, 459) in a dose responsive manner (Adams et al., 1979, 735) and removal of GnRH as in hypothalamic pituitary disconnection leads to a dramatic decrease in serum LH and FSH concentrations (Hamernik et al., 1986; Hamernik and Nett, 1988; Gregg and Nett, 1989). Thus, GnRH is essential for gonadotropin secretion. Similarly, the concentration of GnRH receptor in the pituitary gland has been shown to affect the magnitude of gonadotropin secretory response (Laws et al., 1990).

Unlike LH, the pituitary gland does not maintain a large pool of readily releasable FSH and thus the majority of FSH that is secreted (Miller et al., 1977) is newly synthesized (Miller and Wu, 1981). Synthesis and secretion of FSH in ovine pituitary culture is directly proportional to GnRH binding (Brooks, 1991, 109; Miller et al., 1990). Although FSH secretion is induced by GnRH in culture (Huang and Miller, 1980), it appears that FSH is not as tightly linked to GnRH stimulation *in vivo* (Crowder et. al., 1986; Hamernik and Nett, 1988) because pulsatile delivery of GnRH agonist to ewes did not influence serum concentrations of FSH (Herman and Adams, 1990).

III. Ovarian hormones

The ovary produces several hormones including estradiol, progesterone, and inhibin that interact at the level of the hypothalamus and pituitary gland to regulate gonadotropin synthesis and secretion. With regression of the corpus

luteum, serum concentrations of progesterone decrease, allowing LH pulse frequency to increase (Hauger et al., 1977). This decrease in progesterone concentration stimulates the development of ovarian follicles and leads to increased secretion of estradiol (Hauger et al., 1977) and inhibin (Findlay et al., 1990). Before exposure to high levels of LH, estrogen levels predominate; after the LH surge and during the luteal phase of the cycle, progesterone is the major steroid produced. Gonadal hormones can act directly on the ovine pituitary to cause major changes in GnRH binding and gonadotroph responsiveness to GnRH (Miller et al., 1990). These hormones not only regulate the pulse frequency of GnRH from the hypothalamus but also mRNA levels for LHβ, FSHβ, and α-subunit and GnRH receptor in the anterior pituitary gland. The latter modulates the sensitivity of the anterior pituitary gland to GnRH. Each of these hormones will be discussed to examine their effect.

a. Estradiol

The follicular phase of the ovine estrous cycle is characterized by rising circulating levels of estradiol (Baird et al., 1978) and increased concentrations of GnRH in the hypophyseal portal blood (Clarke et al., 1987). Estradiol affects hypothalamic secretion of GnRH and can stimulate the anterior pituitary gland independent of GnRH (Gregg and Nett, 1989; Turzillo et al., 1995). The effects of estradiol at the hypothalamus and anterior pituitary gland will be reviewed.

1. Hypothalamus

At the hypothalamus, estradiol has a positive effect, decreasing amplitude but increasing the frequency of pulses of GnRH released from the median eminence (Clarke and Cummins, 1985; Caraty et al., 1989; Clarke, 1988; Moenter et al., 1990). According to Evans et al. (1995), estradiol induces secretion of significant amounts of GnRH between pulses and alters the shape of GnRH pulses by reducing the slope of the rising and falling phases of each pulse. These findings lead to the conclusion that during the pre-surge period in the ewe, estradiol induces a qualitative change in the pattern of GnRH release in addition to stimulating GnRH pulse frequency and reducing pulse amplitude (Evans et al., 1995). Thus, increasing concentrations of estradiol stimulate hypothalamic secretion of GnRH during the pre-ovulatory period (Moenter et al., 1990) which will directly affect the actions of the pituitary gland.

2. Pituitary Gland

The responsiveness of the pituitary gland to GnRH is modulated by the number of GnRH receptors on the plasma membrane of the gonadotrophs and can be monitored by measuring serum concentrations of LH (Nett et al., 1981). Therefore, by stimulating an increase in GnRH receptor concentration, estradiol increases the sensitivity of the pituitary gland prior to the pre-ovulatory surge of LH (Reeves et al., 1971; Gregg et. al., 1990; Miller et al., 1990) resulting in an increase in serum levels of LH. Using the hypothalamic pituitary disconnection model to remove GnRH from the system it has been shown that estradiol acts at

the level of the anterior pituitary gland to increase GnRH receptor synthesis and numbers of GnRH receptor at certain phases of the reproductive cycle (Crowder and Nett, 1984; Colin et. al., 1996).

Although GnRH is necessary for the secretion of gonadotropins, estradiol alone can directly increase the number of GnRH receptors in the pituitary in sheep (Gregg and Nett, 1989) and hypogonadal mice (Naik et al., 1985a). Nett and Naik found an additive effect of GnRH on estradiol-induced increase in GnRH receptor (Nett et al., 1984; Naik et al., 1985a).

3. mRNA for LHβ

Rather than causing a simple increase or decrease of gonadotropins, estradiol has a triphasic effect on mRNA for gonadotropins (Herring et al., 1991). First, during the first few hours of estradiol treatment mRNA encoding α-, LHβ- and FSHβ-subunits decrease (Herring et al., 1991). During the second phase estradiol has been shown to increase mRNA for gonadotropin subunits (Leung et al., 1988; Landefeld et al., 1985). It has been shown that mRNA for β-subunits of gonadotropins decreases and mRNA for α-subunit remains unchanged during estradiol positive feedback in OVX ewes (Hamernik and Nett, 1988). Finally, the third phase is characterized by a negative effect of estradiol as demonstrated by chronic treatment of OVX ewes with estradiol which decreases the amounts of mRNA for LHβ (Nilson et al., 1983; Landefeld and Kepa, 1984; Herring et al., 1991). Nilson et al. suggest that estradiol suppresses the accumulation of

mRNAs for α-subunit and LHβ-subunits indicating that glycoprotein hormone assembly may not be limited solely by the rate of accumulation of the β-subunits (Nilson et al., 1983)

Although it would seem logical that a decrease in mRNA subunits would result in a decrease in the pituitary content of gonadotropins, Herring et al. did not find a correlation between pituitary gland content of LH and FSH and subunit mRNAs (Herring et al., 1991). This suggests that the continued high concentration of estradiol may cause a post-transcriptional defect preventing gonadotropin synthesis (Herring et al., 1991). In contrast to Herring et al., (1991), Di Gregorio and Nett (1995) found that estradiol decreased pituitary content of LH and steady-state levels of mRNA for LHβ subunit but serum concentrations of LH were not affected (DiGregorio and Nett, 1995). Thus, it appears that estradiol does not influence the GnRH-stimulated mechanism that results in gonadotropin secretion, although estradiol may negatively effect gonadotropin subunit synthesis.

4. mRNA for FSHβ

FSH does not appear to be as responsive to estradiol as LH. Estradiol decreases steady-state levels of FSHβ mRNA *in vitro* (Counis et al., 1983; Phillips et al., 1988; Alexander and Miller, 1982) but steady-state levels remain unchanged *in vivo* (Di Gregorio and Nett, 1995). Inhibition of transcription appears to be the primary event leading to decreased steady-state levels of

subunit mRNAs and FSH secretion *in vitro* (Miller et al., 1990). After treatment with estradiol, serum concentrations and pituitary content of FSH decreased (Di Gregorio and Nett, 1995).

5. mRNA for α-subunit

Since the gonadotropins are heterodimeric requiring both an α and β subunit to complete the protein, it is important to examine the effects of estradiol on the α subunit. In the absence of GnRH *in vitro*, estradiol inhibits α-subunit gene transcription (Phillips et al., 1988) while *in vivo*, steady-state levels of mRNA for α-subunit in sheep were unchanged by estradiol during a 48 hour treatment (Di Gregorio and Nett, 1995). However, chronic treatment of OVX ewes with estradiol lowers content of α-subunit in the pituitary gland (Nilson et al., 1983; Landefeld et al., 1984; Hall and Miller, 1986). Thus, with long term exposure to estradiol, transcription of α-subunit could be a rate-limiting step in the synthesis of gonadotropins.

6. mRNA for GnRH receptor and GnRH receptor concentration

The concentration of GnRH receptor on the plasma membrane of the gonadotroph is a primary determinant of GnRH responsiveness; therefore, it is important to study how estradiol affects GnRH receptor concentration. Using a hypogonadal model to remove hypothalamic input from the system, it has been shown that estradiol can increase GnRH receptor by direct action on the pituitary

gland in sheep (Gregg and Nett, 1989) and mice (Naik et al., 1985a). Estradiol acts directly at the pituitary gland to increase numbers of GnRH receptor *in vivo* in sheep (Moss et al., 1981; Nett et al., 1981; Crowder and Nett, 1984; Khalid et al., 1987; Clarke et al., 1988; Clarke et al., 1989; Turzillo and Nett, 1995), cattle (Schoenemann et al., 1985), mice (Naik et al., 1985), rats (Clayton et al., 1982; Menon et al., 1985) and monkeys (Adams et al., 1981). Estradiol also act *in vitro* in a dose dependent manner to increase numbers of GnRH receptors (Miller and Huang, 1985; Gregg and Nett, 1989; Gregg et. al., 1990; Laws et al., 1990b).

The increase in GnRH receptor induced by estradiol is dependent on both mRNA transcription and protein synthesis suggesting that estradiol induces expression of the GnRH receptor gene (Gregg et al., 1990). This has been confirmed by Sealfon et al., (1990b), Miller et al. (1993) and Turzillo and Nett (1995).

Experimental treatment of gonadotrophs in culture will cause the number of GnRH receptors in the plasma membrane (Marian and Conn, 1983) to vary. In sheep, receptor numbers range from 500 receptors per cell when treated with mid-cycle concentrations of progesterone (Laws et. al., 1990), to 15,000 to 20,000 receptors, after 48-hour exposure to estradiol-17β (Gregg et. al., 1990; Laws et. al., 1990; Gregg et. al, 1991). In summary, estradiol induces synthesis of GnRH receptor in ovine anterior pituitary cells (Gregg et al., 1990; Sealfon et al., 1990; Laws et al., 1990; Wu et al., 1994) and this increase appears to be

physiologically important to increase numbers of GnRH receptors during the pre-ovulatory LH surge (Crowder and Nett, 1984).

7. LH and FSH Secretion

Estradiol regulation of gonadotropin secretion is complicated in that it's not purely stimulatory or inhibitory. Copping and Malvin (1976) reported estradiol has a biphasic effect on LH secretion meaning that initially estradiol has a negative effect on LH secretion (Nett et al., 1974) as well as pituitary responsiveness to GnRH (Yen et al., 1974) followed by a positive feedback effect. More recently Herring et al. (1991) described estradiol as triphasic, first decreasing serum concentrations of LH for a few hours, then increasing serum LH levels and depleting pituitary gland stores of LH for the next 10 hours (Crowder and Nett, 1984; Schoenemann et al., 1985; Leung et al., 1988) followed by decrease in serum LH levels that appears to remain constant (Herring et al., 1991) accompanied by a slight recovery of pituitary LH levels (Leung et al., 1988; Landefeld et al., 1985). The response of FSH to estradiol exhibits a triphasic response similar to LH but with a much smaller amplitude (Herring et al., 1991).

In OVX ewes (Karsch et al., 1980; Moss et al., 1981) and castrated rams (Schanbacher and Ford, 1977; Parrot and Davies, 1979), long-term treatment with estradiol decreases secretion of LH and FSH, and suppresses pituitary content of gonadotropins (Moss et al., 1981). Pituitary content of LH is lower during anestrus than during the breeding season in intact ewes (Roche et al.,

1970) and in OVX ewes treated chronically with low levels of estradiol (Nett, 1983). Estradiol decreases FSH secretion (Phillips et al., 1988; Alexander and Miller, 1982).

b. Progesterone

When released from the corpus luteum into circulation progesterone exerts it's biological effects at both the hypothalamus and pituitary gland. Progesterone has a negative feedback effect on the hypothalamus causing a decrease in the pulse frequency of GnRH secretion thereby reducing the secretion of gonadotropins LH and FSH into circulation. During the luteal phase and pregnancy higher progesterone levels inhibit GnRH secretion. After luteolysis/parturition progesterone declines allowing an increase in LH secretion (Goodman, 1988).

Progesterone may act independently on the hypothalamus but requires the presence of estradiol to act on the anterior pituitary gland. In intact ewes progesterone suppressed serum concentrations of LH (Moss et al., 1981) suggesting estradiol must be present for progesterone to function. Progesterone's inhibitory effect on LH secretion (Pelletier and Thimoier, 1975) is not mediated via receptors for GnRH or pituitary concentrations of LH, suggesting an influence at higher neural centers (Moss et al., 1981) i.e. the hypothalamus. While the studies above utilize an *in vivo* approach the effects of progesterone to decrease GnRH receptor numbers has also been shown in vitro (Laws et al., 1990a). Progesterone decreases the responsiveness of ovine

cultures to GnRH by approximately 70% (Batra and Miller, 1985) and decreases GnRH binding (Laws, 1990, 725).

Progesterone's inhibition of LH release is also influenced by inhibin. Progesterone can completely inhibit the sensitizing action of inhibin on GnRH-stimulated LH secretion but its inhibitory action is dependent on the presence of estradiol *in vivo* (Batra and Miller, 1986; Turzillo, unpub, 2). Progesterone totally counteracted inhibin induction of GnRH binding and GnRH-stimulated LH secretion *in vitro* (Laws et al., 1990a). In the absence of inhibin (which increases GnRH-stimulated LH release), progesterone decreased GnRH binding but did not affect GnRH stimulated LH release (Laws et al., 1990) indicating that progesterone is not affecting LH release directly but rather attenuates the effects of inhibin.

Progesterone increases pituitary concentrations of LH and FSH, decreases mRNA for FSHβ and α-subunit but does not affect mRNA for LHβ (DiGregorio and Nett, 1995). Estradiol and progesterone can act directly at the pituitary gland of the ewe to regulate steady-state levels of gonadotropin subunit mRNAs independent of GnRH (Di Gregorio and Nett, 1995, 166). However, the effects of progesterone at the pituitary gland require other gonadal inputs such as estradiol (Batra et al., 1986). In contrast progesterone can still apparently act at the hypothalamus (Gasc and Baulieu, 1988; Clarke and Cummins, 1984) in OVX animals. Progesterone has different affects on GnRH receptor *in vivo* and *in vitro*. Chronic administration of progesterone to OVX ewes has no effect on

GnRH receptor (Moss et al., 1981; Hamernik et al., 1987) *in vivo* however this may be because there is no estradiol present. *In vitro*, progesterone decreased the number of GnRH receptor (Laws et al., 1990; Sealfon et al., 1990; Wu et al., 1994) and mRNA for GnRH receptor (Wu et al., 1994). Additionally, progesterone overcomes the positive effect of estradiol and inhibin in combination to decrease GnRH receptor by approximately 87% (Wu et al., 1994). However, it is important to point out that in vitro experiments do indicate that progesterone is active in the absence of estradiol.

Progesterone negatively regulates gonadotropin secretion by decreasing the pulse frequency of GnRH released from the hypothalamus (Moss et al., 1981; Karsch et al., 1987). Thus, gonadotroph responsiveness to GnRH, which is mediated by the number of GnRH receptors on the plasma membrane, effects gondadotropin secretion. During the luteal, phase LH secretion is low most likely due to a lower concentration of GnRH receptor on the plasma membrane (Laws et al., 1990). At luteolysis, when serum progesterone levels decrease due to regression of the corpus luteum, the negative feedback effect is removed and amounts of mRNA for GnRH receptor begin to increase (Turzillo et al., 1994) This should result in a rise in serum levels of LH punctuated by the pre-ovulatory surge in the follicular phase. Hamernik et al. (1995) suggested that the effects of progesterone on GnRH receptor expression may result from 1) decreasing serum concentrations of progesterone; 2) increasing serum concentrations of estradiol (Batra et al., 1986a; Batra et al., 1986b) and /or inhibin; 3) increased secretion of GnRH; or 4) a combination of these (Hamernik et al., 1995).

c. Inhibin

Inhibin, a protein hormone, is produced by the granulosa cells in females and, has been shown to be a primary regulator of FSH synthesis and secretion. Inhibin can inhibit FSH release (Gregg et al., 1990) from the pituitary without altering LH release and is partially responsible for the differential release of LH and FSH from the pituitary (Brooks et al., 1992; Kirk et al., 1994). Porcine and bovine follicular inhibins decrease FSH secretion in rat pituitary cultures (Fukuda et al., 1986; Gregg et al., 1990) and ovine pituitary culture (Miller, 1988; Miller et al., 1990; Gregg et al., 1991).

To further support this in vitro data (Mercer et al., 1987) demonstrated inhibin decreases FSHβ mRNA levels at the pituitary gland in sheep. Although inhibin has a significant effect of FSH secretion and FSHβ mRNA; steady-state levels of α-subunit mRNA and LHβ remain unchanged (Mercer et al., 1987). Therefore, since steady-state levels of α mRNA are unaffected by inhibin (Miller, 1988), the decrease in FSH secretion should be dependent entirely upon decreased transcription of FSHβ mRNA levels unless regulation occurs at translation or post-translationally.

Inhibin, like estradiol, may increase GnRH receptor numbers during the pre-ovulatory period. Miller et al. (1990) demonstrated that inhibin causes a 6.6-fold increase in GnRH binding above control indicating an increase in numbers of GnRH receptor in the pituitary gland. Also, Gregg et al. (1990) showed inhibin

causes an increase in GnRH receptor number. Further, using Scatchard analysis, Gregg et al. (1991) showed that inhibin doubled the number of GnRH receptor. Thus, sensitization of the pituitary gland in vivo results from an increase in receptors.

Treatment with estradiol and inhibin causes the greatest increase in pituitary sensitivity to GnRH and analogs (Miller and Huang, 1985; Laws et al., 1990a; Laws et al., 1990b; Gregg et al., 1990; Gregg et al., 1991). Gregg et al. (1991) concluded that these hormones increase the number of GnRH receptor via separate mechanisms, one by a plasma membrane receptor and the other by an intracellular receptor.

The effects of inhibin to increase GnRH receptor numbers in vitro has led investigators to suggest that both estradiol and inhibin cooperate to increase pituitary gland sensitivity to GnRH during the pre-ovulatory period in (Findlay et al., 1990).

In addition to being secreted into circulation from the follicle, the anterior pituitary gland of rats synthesizes both the α and β subunits of inhibin, although secretion of bioactive inhibin by the anterior pituitary has yet to be demonstrated (Roberts et al., 1992). Thus, there is potential for an autocrine or paracrine effect of inhibin produced by the anterior pituitary gland to induce synthesis of GnRH receptor or effect mRNA for gonadotropin subunits.

VII. The Problem—Role of GnRH and Estradiol

When secreted in a pulsatile mode, GnRH increases concentrations of mRNA for GnRH receptors, presumably via an increase in the rate of transcription, which in turn leads to synthesis of new GnRH receptors which are inserted into the cell membrane. However, if GnRH is administered continuously, as in the case of clinical treatment for endometriosis, uterine fibroid tumors, and breast cancer, GnRH receptors are internalized more rapidly than they are replenished and the pituitary gland becomes refractory to stimulation by GnRH. In most instances it is probably degraded. What is not known is whether this homologous desensitization is mediated only by receptor internalization or whether continuous treatment with GnRH also affects expression of GnRH receptor gene.

Another important modulator of GnRH receptor synthesis is estradiol. Estradiol is a steroid hormone that regulates GnRH receptor synthesis by binding to intracellular receptors, which in turn, interact with DNA. Previous experiments have shown that treatment with exogenous estradiol increases the steady-state levels of mRNA for GnRH receptors and concentration of GnRH receptors.

So, although GnRH and estradiol regulate expression of GnRH receptor, they appear to use different pathways that are probably mediated via different response elements on the DNA. Because these two hormones influence GnRH receptors through independent pathways, it is hypothesized that: One, continuous infusion of GnRH will decrease steady-state levels of mRNA encoding GnRH receptor. And two, since the effects of estradiol are regulated

by a receptor different from that for GnRH, we also hypothesize estradiol will override the negative effects of continuous GnRH infusion on mRNA encoding GnRH receptor.

CHAPTER TWO

REGULATION OF GnRH RECEPTOR mRNA

A. Abstract

GnRH serves as a homologous regulator of its own receptor and can either increase (pulsatile delivery) or decrease (continuous delivery) number of GnRH receptors on gonadotrophs. Estradiol also regulates numbers of GnRH receptor, but always in a positive manner. Because estradiol acts via intracellular receptors, and GnRH exerts its effects through a membrane receptor, these two hormones appear to regulate GnRH receptor by different mechanisms. The objective of this experiment was to test the hypothesis that estradiol will override the negative effect of continuous infusion of GnRH on GnRH receptor gene expression and stimulate de novo GnRH receptor synthesis. OVX ewes were administered GnRH (10μg/h, n = 10), to down-regulate GnRH receptor, or saline (n = 10) continuously via subcutaneous Alzet osmotic mini-pumps for 136 h. At 124 h, 5 ewes in each group were administered estradiol (25 μg IM) and anterior pituitary glands were collected 16 hours later. Blood samples were collected every 4 hours for 120 hours and then every 30 min for the duration of the study. GnRH caused an initial increase in circulating levels of LH with peak levels occurring approximately 5 ½ hours after

beginning infusion, after which concentrations of LH decreased to and remained at pre-treatment levels. Circulating concentrations of LH increased approximately 10 hours after treatment with estradiol in ewes administered saline, whereas, circulating concentrations of LH in ewes treated with a continuous infusion of GnRH increased 4 ½ hours earlier—a more rapid response.

Treatment with GnRH and estradiol reduced levels of mRNA for LHβ ($p<0.01$). Likewise, treatment with GnRH alone reduced mRNA for LHβ ($p<0.005$). Alpha-subunit levels did not change in response to either treatment with GnRH or estradiol. Compared to saline-treated controls, treatment with GnRH alone decreased the amount of FSHβ mRNA ($p <0.05$). Treatment with estradiol also caused a decrease in FSHβ mRNA ($p < 0.01$). Levels of FSHβ mRNA in ewes treated with both GnRH and estradiol were significantly lower than the control group ($P<0.0005$).

Neither treatment with GnRH or estradiol affected pituitary concentration of LH or FSH relative to controls. Treatment with estradiol or GnRH plus estradiol decreased pituitary content of FSH relative to treatment with GnRH alone ($p<0.025$).

Compared to saline-treated controls, treatment with GnRH alone decreased the amount of mRNA for GnRH receptor ($p <0.01$) and concentration of GnRH receptor ($p<0.05$) while treatment with estradiol caused a nearly 2-fold increase in GnRH receptor mRNA ($p < 0.05$) and GnRH receptor ($p<0.01$) .

Levels of mRNA for GnRH receptor and numbers of GnRH receptors in ewes treated with both GnRH and estradiol were comparable to those in the control group indicating that estradiol was able to override the negative effect of GnRH by stimulating an increase in mRNA for GnRH receptor and GnRH receptor concentrations. Thus, from these data we conclude that although the gonadotroph becomes refractory to GnRH during homologous desensitization, this desensitization does not affect the cell's ability to respond to estradiol.

B. Introduction

At the level of the anterior pituitary gland, binding of GnRH to GnRH receptor on gonadotrophs initiates the cascade of events necessary for the synthesis and secretion of LH and FSH (Schulling et al., 1976; Braden and Conn, 1991). Sensitivity of the pituitary gland to GnRH is dependent on the concentration of GnRH receptors on the plasma membrane. GnRH and estradiol regulate concentrations of GnRH receptor (Clarke et al., 1988), and therefore, these hormones impact the sensitivity of gonadotrophs and ultimately help regulate gonadotropin secretion. Effects of stradiol on gonadotrophs have been studied in the absence of hypothalamic input; however, it has not been determined if estradiol can override the negative effects of a continuous infusion of GnRH.

In the natural state, cycling ewes receive pulsatile GnRH from the hypothalamus to maintain normal anterior pituitary activity (Karsch et al., 1987; Turzillo et al., 1995a) and continued GnRH stimulation is required to maintain

steady-state concentrations of GnRH receptor (Nett et al., 1984; Hamernik et al., 1986; Sakurai et al., 1996), and mRNA for α-, FSHβ- and LHβ-subunit (Hamernik and Nett, 1988a; Windle et al., 1990) and mRNA for GnRH receptor (Turzillo et al., 1995; Sakurai et al., 1997). Numbers of receptors is correlated with GnRH-induced LH release (Wise et al., 1984). Therefore, an increase in GnRH receptor in the presence of GnRH leads to an increase in serum concentrations of LH (Gregg and Nett, 1989; Gregg et al., 1991).

GnRH is a homologous regulator of its own receptor (Nett et al., 1981; Khalid et al., 1987; Turzillo et al., 1995a) because depriving the pituitary gland of GnRH by hypothalamic-pituitary-disconnection results in decreased numbers of GnRH receptor (Clarke et al., 1987a; Gregg and Nett, 1989; Nett, 1990; Turzillo et al., 1995) which can be restored to control values by replacement of pulsatile GnRH *in vivo* (Clarke et al., 1987a; Hamernik and Nett, 1988a) and *in vitro* (Kaiser et al., 1993). Pulsatile release of GnRH increases both concentrations of mRNA for GnRH receptor and numbers of receptors (Turzillo et al., 1995a). In contrast, continuous infusion of GnRH leads to desensitization of gonadotrophs (Conti et al., 1977; Nett et. al., 1981; Crowder et al., 1986; Mason et al., 1994). This desensitization is characterized by a decrease in the number of receptors (Nett et al., 1981) by approximately 50% (Crowder et al., 1986) and can be identified by measuring serum levels of LH (Nett et al., 1981).

Because endocrine communication between the hypothalamus and pituitary are prevented in the HPD model, it has been shown that estradiol

affects expression of the GnRH receptor gene *in vivo* in the absence of GnRH (Turzillo et. al, 1995). Treatment with estradiol consistently increases numbers of GnRH receptors *in vivo* (Moss et al., 1981; Menon et al., 1985; Schoenemann et al., 1985; Gregg and Nett, 1989; Turzillo et al., 1994) and *in vitro* (Laws et al., 1990b; Gregg et al., 1990; Wu et al., 1994).

Based on current research, both GnRH and estradiol contribute to increased concentrations of mRNA for GnRH receptor (Brooks et al., 1993; Turzillo et al., 1994) and maximal expression of GnRH receptor (Crowder and Nett, 1984). So, although estradiol increases the number of GnRH receptors, the mechanism of this increase in unclear. Does estradiol decrease the rate of receptor internalization, increase the rate of recycling, or increase transcriptional rates? If estradiol is able to increase GnRH receptor synthesis, will a continuous infusion of GnRH cause GnRH receptor to internalize faster than estradiol can stimulate new synthesis? The potential for estradiol to override the negative effect of continuous infusion of GnRH on expression of GnRH receptor gene and stimulation of *de novo* GnRH receptor synthesis was outlined with the following objectives: 1) to characterize changes in steady-state concentrations of mRNA for GnRH receptor and numbers of GnRH receptors after down-regulation of anterior pituitary gland with a continuous infusion of GnRH, and 2) to measure effects of estradiol on concentrations of GnRH receptor mRNA and numbers of GnRH receptors after anterior pituitary gland desensitization by GnRH.

C. Materials and Methods

I. Animals and Treatments

Sexually mature, Western range ewes that had been OVX for at least 6 weeks were used in this experiment. Twenty ewes were randomly assigned to four treatment groups. Each treatment group contained five ewes. Ten ewes were administered saline and ten ewes were administered GnRH (10 μg/hr) continuously via subcutaneous Alzet osmotic mini-pumps (alza Scientific Products, Palo Alto, CA) for 136 hours. At 124 hours 5 ewes receiving saline were given a 25 mg intramuscular injection of estradiol-17β (Sigma, St. Louis, MO) dissolved in safflower oil. Also, at 124 hours, 5 ewes receiving GnRH were given an intramuscular injection of 25 mg of estradiol. At 136 hours, after anesthesia with sodium pentobarbital (29 mg/kg BW, iv.) and exsanguination, anterior pituitary glands were collected from all 20 OVX ewes. Pituitaries were divided midsagitally, immediately frozen on solid CO_2, and stored at -80°C. Jugular blood samples were collected every 4 hours for 120 hours and then every 30 minutes for 16 hours. All procedures involving animals were approved by the Colorado State University Animal Care and Use Committee, and were in compliance with NIH guidelines.

II. Analysis of mRNA

Polyadenylated RNA was prepared from one-half of each pituitary gland (Badley et al., 1988). The integrity of this RNA was verified with Northern blot

analysis of mRNA for GnRH receptor (Turzillo et al., 1994). Steady-state concentrations of mRNA encoding GnRH receptor (Nett et al., 1981), LHβ-subunit and α-subunit (Nett et al., 1990), and FSHβ-subunit (Hamernik et al., 1987) were quantified. Briefly, 1.5 μg of poly A+ RNA were immobilized in duplicate on a nylon membrane (Hybond; Amersham, Arlington Heights, IL), cross-linked by exposure to ultraviolet light (Stratagene, La Jolla, CA), and probed with cDNAs for ovine GnRH receptor (Nett et al., 1981), LHβ (Nett et al., 1990), FSHβ (Hamernik et al., 1987), and α-subunit (Turzillo et al., 1994). The cDNAs were radiolabeled using the random hexamer priming method (Boehringer Mannheim, Indianapolis, IN), and hybridization was performed at 42°C for 24 h in hybridization solution [100% deionized formamide, 10% SDS, denatured, sheared salmon sperm DNA (10mg/ml), 10X Pipes-NaCl-EDTA, nanopure water]. After hybridization, the final wash was in 0.5X SSC (750 mM NaCl and 75 mMNa citrate), 0.1% SDS at 65°C. The nylon membrane was exposed to Hyperfilm MP (Amersham, Arlington Heights, IL) for 4 days (FSHβ/GnRH receptor), 7.5 h (LHβ), or 8 h (α-subunit). After each autoradiography, the membrane was stripped of the respective cDNA as previously described (Turzillo et al., 1994) and reprobed with radiolabeled poly dT (18mer) which was end-labeled with [^{32}]PgATP (3000 Ci/mmol; Amersham) using T4 polynucleotide kinase (New England Biolabs, Beverly, MA). Hybridization to radiolabeled dT was performed at 30°C for 1 h in 5x SSC, 10mM $NaHPO_4$, 1mM NaH_2PO_4 and 0.12% sarkosyl. Membranes were washed in 2X

SSC at 30°C and exposed to film for 20 min (Juengel et al., 1994). Autoradiographs were analyzed with the NIH 1.52 Image Analysis Program. Concentrations of cDNA were normalized to the amount of poly A+ RNA in each sample and expressed as arbitrary units.

III. Hormone Assays

Circulating concentrations of LH were determined by radioimmunoassay (Niswender et al., 1969) using NIH-oLH-S24 as reference preparation. Mean limit of detection, intraassay and interassay coefficient of variation (CV) were 127 pg/ml, 11.4% and 11.4%, respectively. Circulating concentrations of FSH (L'Hermite et al., 1972) were determined by radioimmunoassay. Mean limit of detection, intraassay and interassay coefficient of variation (CV) were 6.0 ng/ml, 10.3%, and 10.8%, respectively.

IV. Measurement of Receptors for Gonadotropin-Releasing Hormone

Numbers of GnRH receptors were quantified using a standard curve technique described previously (Nett et al., 1981). Briefly, the concentration of GnRH receptor in a pool of bovine pituitary membranes was determined by Scatchard analyses. A standard curve was generated by incubating increasing quantities of the membrane pool with 0.2 nM [^{125}I]D-Ala^{6}-GnRH-Pro^{9}-ethyl-amide ([^{125}I]D-Ala^{6}). Partially purified membranes prepared from each experimental pituitary gland were incubated with 0.2 nM [^{125}I]D-Ala^{6} in assay buffer [1.21g Tris

Base 10 mM, 147mg $CaCl_2$ 1 mM, 1g BSA 0.1%] for 4 hours at 4°C. At the end of the incubation, tubes were centrifuged immediately after the addition of 3 mL of ice-cold assay buffer. Amounts of specifically bound [^{125}I]D-Ala^6 in the pellets were compared to the standard curve, and the number of GnRH receptor in each sample was calculated. All samples were quantified in a single assay.

V. *Statistical Analysis*

Data were analyzed using 1-way Analysis of Variance and when significant treatment effects were observed, means were separated using Least Significant Differences. Pearson correlation coefficient (r) was computed to describe the relationship between concentrations of mRNA for GnRH receptor and numbers of GnRH receptors. Data are presented as mean ± SEM.

D. Results

I. Effect of GnRH on serum levels of LH

GnRH caused serum levels of LH to begin rising immediately, reaching a peak at approximately 5 ½ hours. Then serum levels of LH decreased to remain at levels control ewes for the duration of the experiment (Figure 4). At 124 hours (Figure 5), ½ of the saline treated ewes were given 25 mg of estradiol intramuscularly. This induced an immediate decrease in secretion of LH followed by an increase in secretion of LH beginning approximately 10 hours later. Half of the GnRH treated ewes were also administered estradiol at 124

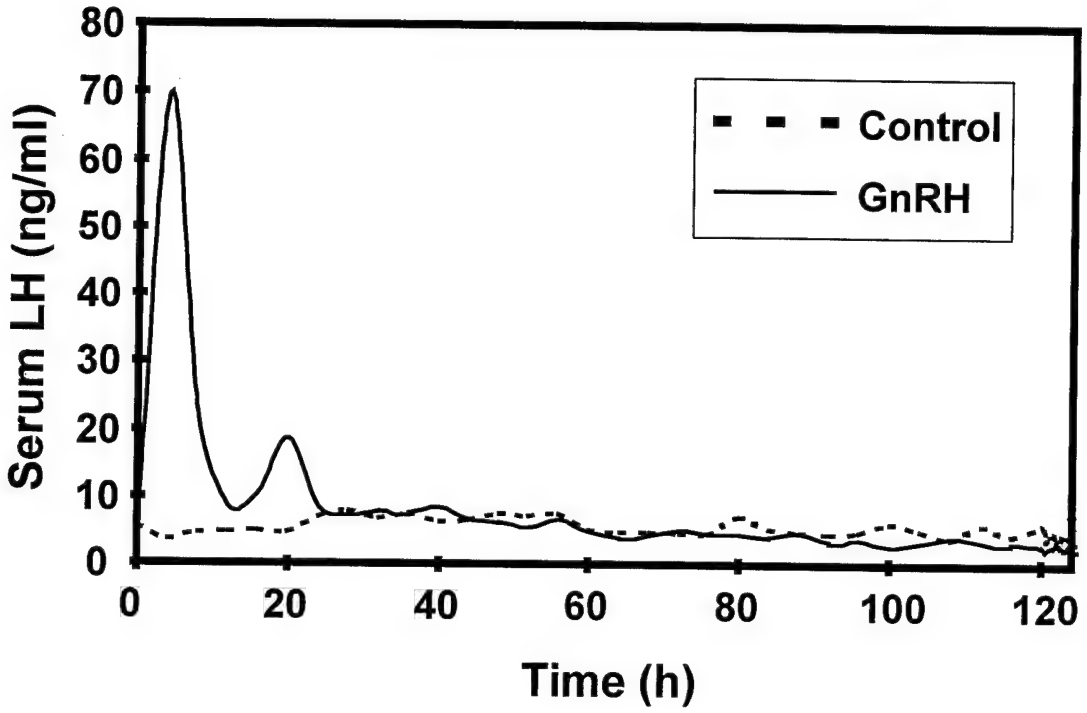

Figure 4. Mean concentrations of LH in serum in OVX ewes. Control ewes (n=10) received saline while 10 ewes received GnRH continuosly via subcutaneous Alzet osmotic mini-pumps for 136 h. At 124 h, 12 h prior to pituitary gland collection, estradiol (25 μg IM) was administered to (n=5 saline treated ewes) and (n=5 GnRH treated ewes). Blood samples were taken every 4 h for 120 h after which sampling frequency increased to every 30 minutes.

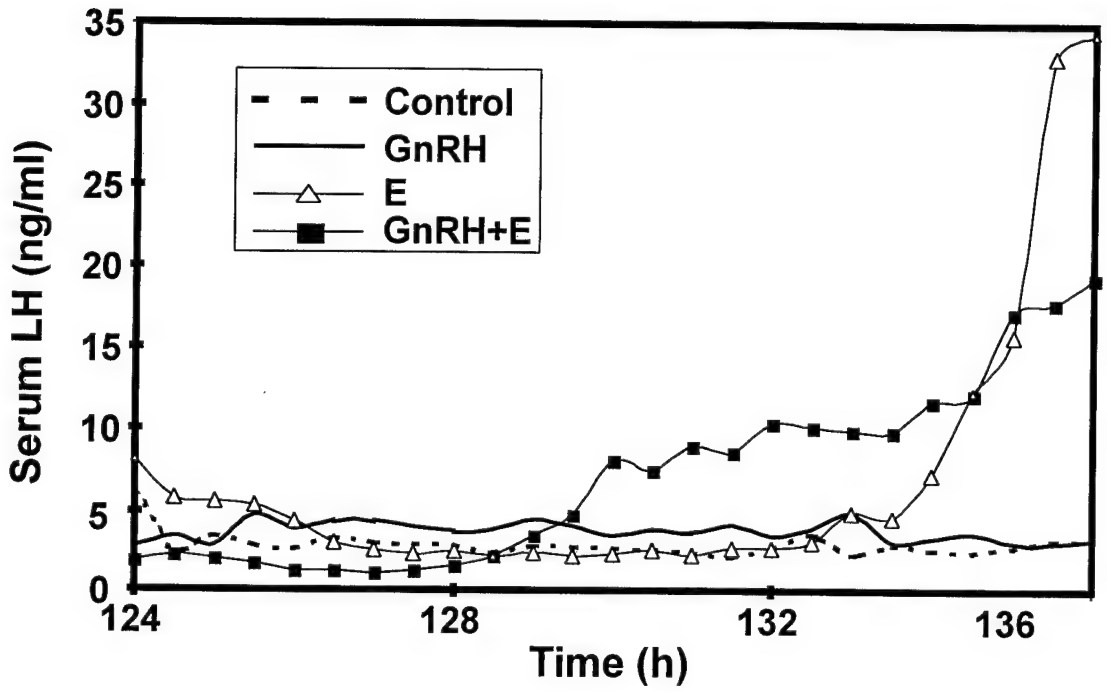

Figure 5. Mean concentrations of LH in serum of OVX ewes from 120 h to 136 h. Control ewes (n=10) received saline, while other ewes were treated continuously with GnRH (n=10). At 124 h, 12 h prior to pituitary gland collection, estradiol (25 mg IM) was administered to saline-treated ewes GnRH-treated ewes. Blood samples were taken every 30 minutes.

hours (Figure 5). There was also a decrease in LH secretion followed by an increase in LH secretion in these ewes in response to estradiol, but it occurred sooner, approximately 4½ hours after the estradiol injection, rather than 10 hours after the estradiol injection as occurred in the saline treated ewes. The magnitude of the release of LH in ewes receiving GnRH plus estradiol was less than in ewes receiving only estradiol. Serum levels of FSH in the treatment groups were not significantly different from control levels (Figure 6).

II. mRNAs for LHβ/FSHβ/α-subunit and pituitary concentrations of gonadotropins

Treatment with GnRH and estradiol (Figure 7) reduced mRNA for LHβ ($p<0.01$) approximately 59% and in the group treated with GnRH alone mRNA for LHβ decreased ($p<0.005$) approximately 62%. Neither treatment with GnRH or estradiol resulted in a significant variation in α-subunit from control ewes. Compared to saline-treated controls, treatment with GnRH alone decreased the amount of FSHβ mRNA ($p <0.05$) approximately 65%. Likewise, treatment with estradiol also caused a decrease in FSHβ mRNA ($p < 0.01$) of approximately 65%. Levels of FSHβ mRNA in ewes treated with both GnRH and estradiol were significantly lower than the control group ($p<0.005$) by 91%.

Estradiol induced a decrease in pituitary concentration of LH ($p<0.05$) by 72% relative to GnRH treatment (Figure 8). Likewise, estradiol induced a decrease in FSH ($p<0.02$) by 87% relative to GnRH treatment.

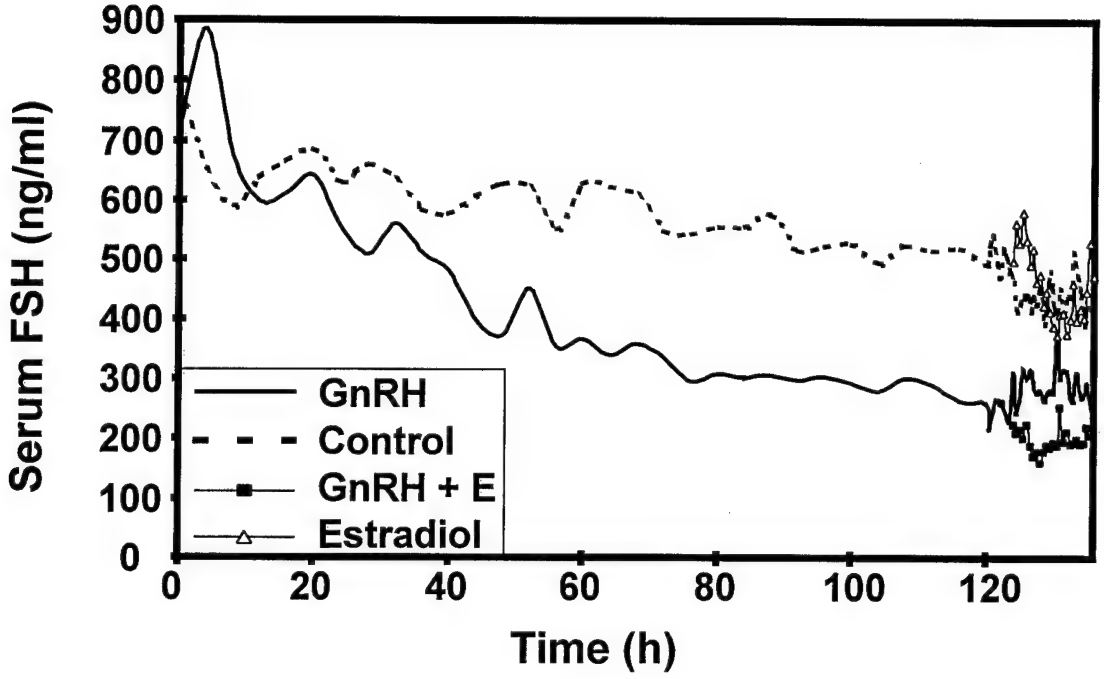

Figure 6. Mean concentrations of FSH in serum of OVX ewes. Control ewes (n=10) received saline via subcutaneous Alzet osmotic mini-pumps for 136 h, while treatment groups received GnRH (n=10) via subcutaneous Alzet osmotic mini-pumps for 136 h. At 124 h, 12 h prior to pituitary gland collection, estradiol injection (25 mg IM) was administered to (n=5 saline treated ewes) and (n=5 GnRH treated ewes). Blood samples were taken every 4 h for 120 h after which sampling frequency increased to every 30 minutes.

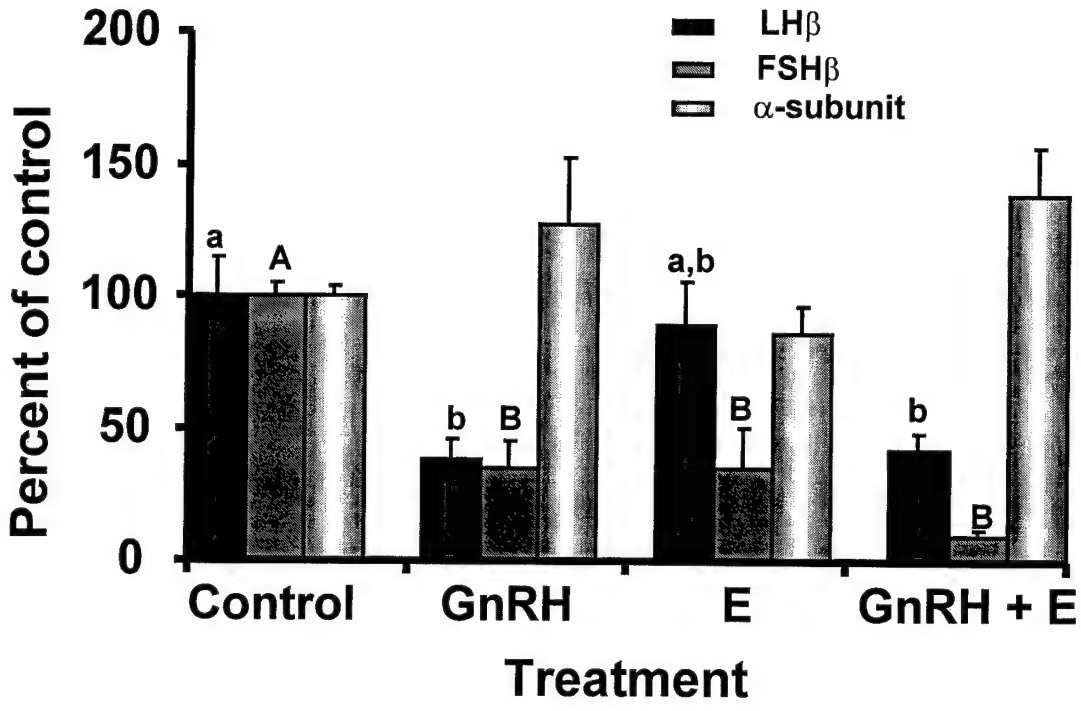

Figure 7. Mean concentrations ± SEM of mRNA for LHβ, FSHβ, and α-subunit in pituitary tissue of OVX ewes (n=5/group). Groups were treated with a continuous infusion of GnRH or saline (n=10). ½ of each group was given a 25 mg IM of estradiol 12 h prior to pituitary gland collection. Significant differences among mean concentrations of mRNA for LHβ, FSHβ, and α-subunit are indicated by letters.

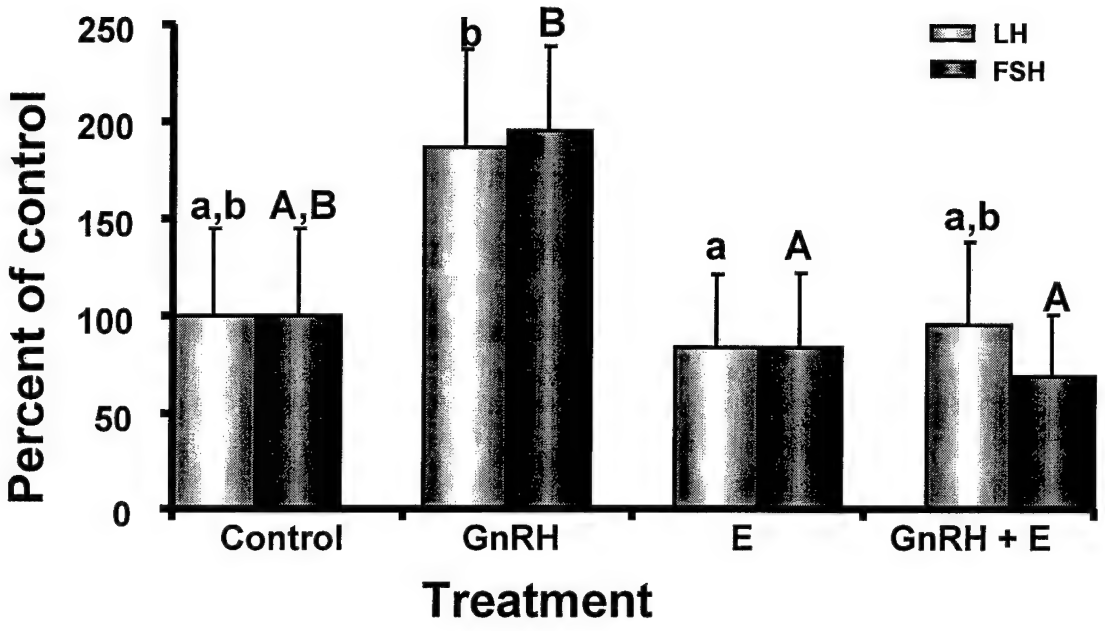

Figure 8 . Mean concentrations ± SEM of LH and FSH in pituitary tissue of OVX ewes (n=5/group). Groups were treated with a continuous infusion of GnRH or saline (n=10). ½ of each group was given a 25 mg IM of estradiol 12 h prior to pituitary gland collection. Significant differences among mean concentrations of LH or FSH are indicated by letters.

III. GnRH receptor mRNA and GnRH receptor concentrations

Figure 9 illustrates mean concentrations of mRNA encoding GnRH receptor and number of GnRH receptor, represented as percent of control values. Relative to control values, levels of mRNA encoding GnRH receptor in ewes treated continuously with GnRH decreased ($P<0.01$) by 48% and concentrations of GnRH receptor decreased ($P<0.05$) by 69%. Also, in comparison to control values, treatment with estradiol increased the amount of mRNA encoding GnRH receptor ($P<0.05$) by 62% and concentrations of GnRH receptor increased ($P<0.01$) by 89%.

Concentrations of mRNA for GnRH receptor in ewes treated with estradiol and GnRH were elevated in this group relative to levels in ewes treated only with GnRH ($P<0.001$) by 44% . Numbers of GnRH receptors in ewes treated with estradiol and GnRH was also elevated relative to the GnRH treated ewes ($P<0.005$) by 111%. However, concentrations of both mRNA encoding GnRH receptor and concentrations of GnRH receptor in ewes treated with GnRH and estradiol were not different from those in controls. Concentrations of GnRH receptor were correlated to levels of GnRH receptor mRNA (0.78 Pearson correlation, $p<0.0001$).

E. Discussion

Continuous GnRH infusion has been shown to down-regulate the anterior pituitary gland decreasing numbers of hypophyseal receptors for GnRH (Nett et al, 1981; Crowder et al., 1986). Therefore, to validate the first premise of our

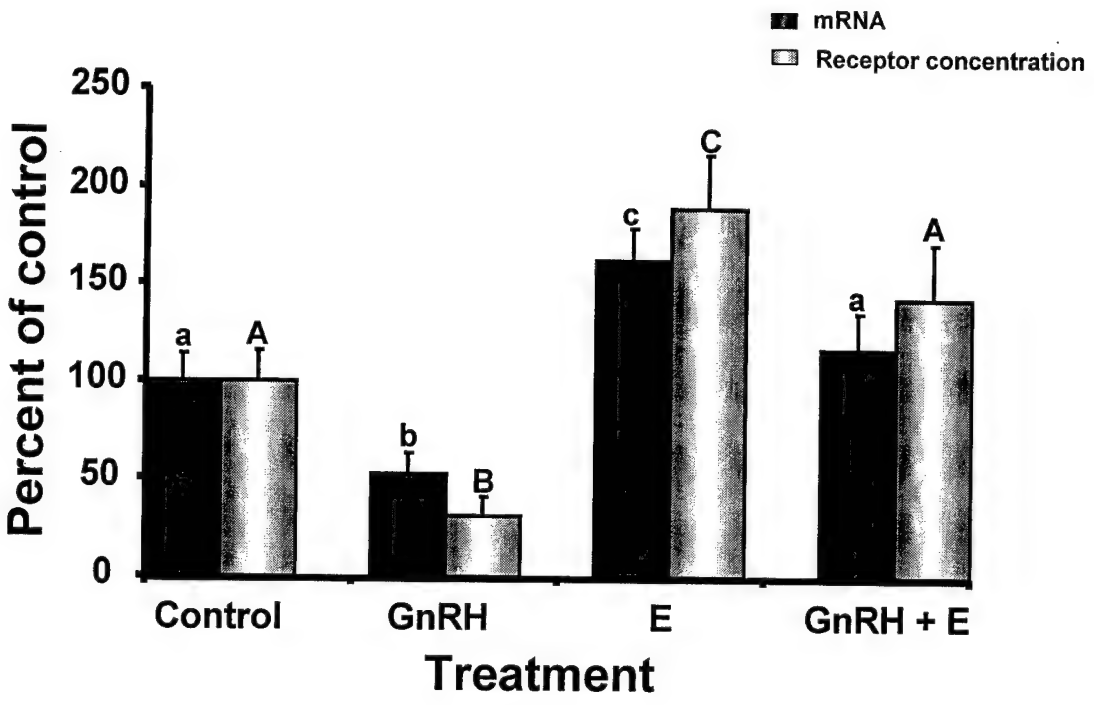

Figure 9. Mean concentrations ± SEM of GnRH receptor and mRNA for GnRH receptor in pituitary tissue of OVX ewes (n=5/group). Groups were treated with a continuous infusion of GnRH or saline (n=10). ½ of each group was given a 25 mg IM of estradiol 12 h prior to pituitary gland collection. Significant differences among mean concentrations of GnRH mRNA or GnRH receptor are indicated by letters.

experimental model, concentrations of serum LH were used to verify that continuous infusion of GnRH caused desensitization of gonadotrophs. As expected, our GnRH treatment induced an LH surge within 5 ½ hours after insertion of implants (Chakraborty et al., 1974; Schuiling et al., 1976; Vizcarra et al., 1997). Following the LH surge, mean concentrations of serum LH in ewes treated with a continuous infusion of GnRH were not different from control serum levels of LH for the duration of the experiment, a previously characterized phenomenon (Nett et al., 1984). Since the GnRH treated ewes continued to be exposed to levels of GnRH that induced a surge of LH, we concluded that gonadotrope desensitization had occurred.

Estradiol has previously been reported to decrease plasma concentrations of LH within 6 hours after treatment (Clarke et al, 1988) followed by an increase in serum concentrations of LH at approximately 16-20 hours (Clarke et al., 1988; Nett et al., 1984). Five of the saline treated ewes that were given estradiol at 124 hours showed a decrease in LH secretion within an hour which was followed by an increase in LH secretion approximately 10 hours later. This matches the effect of estradiol reported by Herring et al. (1991) who found treatment with estradiol decreased serum concentrations of LH from 1-10 hours and then elevated serum levels of LH from 11-22 hours (Herring et al., 1991).

Half of the GnRH treated ewes were administered estradiol at 124 hours. There was also an increase in secretion of LH in these ewes in response to estradiol, but it occurred sooner, approximately 5 ½ hours after the estradiol injection, rather than at 10 hours after the estradiol injection as occurred in the

saline treated ewes. Since estradiol increases the concentrations of GnRH receptors, the ewes receiving a continuous infusion of GnRH were able to respond instantly to an increase in concentrations of GnRH receptors.

Levels of mRNA for FSHβ decreased and it appears that a continuous infusion of GnRH has effects similar to removal of GnRH stimulus through immunoneutralization (Sakurai et al., 1997). Treatment with estradiol also caused a decrease in mRNA for FSHβ. Levels of mRNA for FSHβ in ewes treated with both GnRH and estradiol were significantly lower than the control group indicating an additive negative effect on mRNA synthesis.

Previously, estradiol did not alter mRNA for LHβ (Brooks, 1993), however, treatment with GnRH and estradiol, and treatment with GnRH alone both reduced mRNA for LHβ. Neither treatment with GnRH or estradiol resulted in a significant variation in α-subunit from control ewes which supports earlier data collected by Di Gregorio and Nett (1995).

Estradiol treatment does not alter pituitary LH concentrations (Clarke et al., 1989) as affirmed by results. It appears that FSH release is directly related to the rate of synthesis (Brooks et al., 1992). Our data indicate that continuous GnRH and estradiol treatment decreased gonadotropin synthesis, however, we found pituitary concentrations of FSH increased in response to a continuous infusion of GnRH.

The first objective of this experiment was to characterize changes in steady-state concentrations of GnRH receptor (Nett et al., 1981; Wise et al.,

1984) and mRNA for GnRH receptor (Turzullo et al., 1995; Sakurai et al., 1997). In accordance with previous data, in our experiment relative to control values, levels of mRNA encoding GnRH receptor and concentrations of GnRH receptor in ewes treated continuously with GnRH decreased, thus confirming desensitization is not solely due to receptor internalization.

The second objective was to measure the effects of estradiol on concentrations of GnRH receptor and amounts of mRNA for GnRH receptor after desensitization by GnRH. Previously, estradiol has been shown to increase GnRH receptor concentrations (Moss et al., 1981; Sealfon et al., 1990; Gregg et al., 1990) and increase steady-state levels of mRNA for GnRH receptor (Wu et al., 1994; Turzillo et al., 1994; Hamernik et al., 1995). Again, in direct comparison with previous publications, treatment with estradiol increased concentrations of GnRH receptor (Clarke et al., 1988; Gregg and Nett, 1989) and the amount of mRNA encoding GnRH receptor (Turzillo et al., 1995) confirming that the stimulatory effects of estradiol on numbers of GnRH receptor in sheep occur directly on the pituitary gland because the continuous infusion of GnRH decreases the sensitivity of the pituitary gland to GnRH from the hypothalamus. However, whether estradiol can override the negative affect of GnRH desensitization on the anterior pituitary gland and increase mRNA for GnRH receptor had not been shown.

Our comparison of estradiol and GnRH treated groups to GnRH only treated groups revealed that estradiol increases expression of GnRH receptor gene in the presence of GnRH because concentrations of GnRH receptor mRNA

were elevated in this group relative to levels in ewes treated only with GnRH. GnRH receptor was also elevated relative to the GnRH treated ewes. These data show that estradiol is able to increase synthesis of GnRH receptor faster than a continuous infusion of GnRH can cause receptor internalization.

F. Implications

Hypersecretion of luteinizing hormone has been implicated in infertility and miscarriages in women (Risma et al., 1995) and may lead to cyst formation, ovarian tumorigenesis, and infertility (Risma et al., 1995). Since pulsatile GnRH stimulates the gonadotrophs to release gonadotropins, a continuous infusion of GnRH to desensitize the gonadotrophs and prevent LH secretion is used as a method of clinical treatment. As further examples, GnRH can be administered continuously for clinical treatment for endometriosis, uterine fibroid tumors, and breast cancer. This GnRH treatment can elicit menopausal symptoms which may warrant estrogen therapy (Leather et al., 1993). Although the gonadotroph becomes refractory to GnRH during homologous desensitization, this desensitization does not affect the cell's ability to respond to estradiol. Consequently, the down-regulation of mRNA for GnRH receptor and concentrations of GnRH receptor sought through continuous GnRH treatment can be overridden by exogenous estradiol delivered by estrogen therapy. So, where previously, the treatment of continuous GnRH may have been recorded as ineffective; doctors may now understand that the GnRH treatment was most

likely effective but neutralized by estrogen therapy to counteract menopausal symptoms.

CHAPTER THREE

CONCLUSIONS

As discussed previously the continuous infusion of GnRH caused desensitization of the gonadotrophs. Treatment with estradiol caused concentrations of serum LH to increase in both the saline-treated ewes and in ewes pre-treated with a continuous infusion of GnRH. However, it is important to note the onset of this increase was 5 ½ hours earlier in ewes treated with GnRH. We presume this is because high levels of circulating GnRH from the pumps was readily available as new GnRH receptors were inserted in to the plasma membrane.

Continuous administration of GnRH caused a decrease in the steady-state levels of mRNA encoding GnRH receptor and GnRH receptor concentrations. In contrast, treatment with estradiol induced an increase in levels of mRNA for GnRH receptor and GnRH receptor relative to controls and in the group receiving continuous GnRH. These results support the conclusion that estradiol, acting on the pituitary gland, stimulates transcription of the GnRH receptor gene. Desensitization of the gonadotrophs causing a decrease in mRNA for GnRH receptor and concentrations of GnRH receptors can be overridden by exogenous estradiol. These results support the hypothesis that estradiol may override inhibition of GnRH receptor caused by continuous

exposure of gonadotrophs to GnRH, and suggest that new GnRH receptor are synthesized and inserted into the plasma membrane within 6 h after administration of estradiol.

These data are important because GnRH therapy is used to treat several medical conditions. Hypersecretion of luteinizing hormone has been implicated in infertility and miscarriages in women (Risma et al., 1995) and may lead to cyst formation, ovarian tumorigenesis, and infertility (Risma et al., 1995). Since pulsatile GnRH stimulates the gonadotrophs to release gonadotropins, a continuous infusion of GnRH is used to desensitize the gonadotrophs and prevent the pre-ovulatory LH surge. GnRH can be administered continuously for clinical treatment for endometriosis, uterine fibroid tumors, and breast cancer. This GnRH treatment can elicit menopausal symptoms which may warrant estrogen therapy (Leather et al., 1993). Although the gonadotroph becomes refractory to GnRH during homologous desensitization, this desensitization does not affect the cell's ability to respond to estradiol. Consequently, the down-regulation of mRNA for GnRH receptor and concentrations of GnRH receptor sought through continuous GnRH treatment can be overridden by exogenous estradiol delivered by estrogen therapy. So, where previously, the treatment of continuous GnRH may have been recorded as ineffective; doctors may now understand that the GnRH treatment was most likely effective but neutralized by the estrogen therapy used to counteract menopausal symptoms.

Chapter Four

References

Adams TE, Wagner TO, Sawyer HR, Nett TM (1979) GnRH interaction with anterior pituitary. II. Cyclic AMP as an intracellular mediator in the GnRH activated gonadotroph. *Biol Repro* **21**:735-747

Adams TE, Norman RL, Spies HG (1981) Gonadotropin-releasing hormone receptor binding and pituitary responsiveness in estradiol-primed monkeys. *Science* **213**:1388-1390

Aiyer MS, Chiappa SA, Fink G (1974) A priming effect of luteinizing hormone-releasing factor on the anterior pituitary gland in the female rat. *J Endocrinology* **62**:573

Alexander DC, Miller WL (1982) Regulation of ovine follicle-stimulating hormone hormone β-chain mRNA by 17β-estradiol in vivo and in vitro. *J Biol Chem* **257**: 2282-6

Amos M, Burgus R, Blackwell R, Vale W, Fellows R, Guillemin R (1971) Purification and amino acid composition and N-terminus of the hypothalamic luteinizing hormone releasing factor (LRF) of ovine origin. Biochem. *Biophys. Res. Comm.* **44**: 205-210

Arimura A, Schally AV (1970) Stimulation of release of LH by synthetic LH-RH *in vivo*. I. Comparative study of natural and synthetic hormones. *Endocrinology* **90**:163-68

Aschner B (1912) Uber die funktion der hypophyse. *Arch. Ges. Physiol.* **146**:1-146

Badley JE, Bishop GA, St. John T, Frelinger JA (1988) A simple, rapid method for the purification of poly A + RNA. *Biotechniques* **6**:114-6

Batra SK, Miller WL (1989) Progesterone decreases the responsiveness of ovine anterior pituitary cultures to luteinizing hormone-releasing hormone. *Endocrinology* **117**: 1436-1440.

Batra SK, Britt JH, Miller WL (1986a) A direct pituitary action of progesterone on basal secretion of follicle-stimulating hormone in ovine cell culture: dependence on ovaries *in vivo*. *Endocrinology* **119**: 1929-1932

Batra SK, Miller WL (1986b) Progesterone antagonizes the ability of porcine

ovarian inhibin to sensitizxe oving pituitary cell culture to luteinizing hormone-releasing hormone: dependence on ovaries *in vivo.* *Endocrinology* **119**:1933-38

Beato M (1989) *Cell* **56**:335-44

Blue BJ, Pickett BW, Squires EL, McKinnon AO, Nett TM, Amann RP, Shiner KA (1991) Effect of pulsatile or continuous administration of GnRH on reproductive function of stallions. *J Reprod Fertil Suppl* **44**:145-154

Bokar JA, Keri RA, Farmerie TA, Fenstermaker RA, Andersen B, Hamernik DL, Yun J, Wagner T, Nilson JH (1989) Expression of the Glycopritein Hormone α-Subunit Gene in the Placenta Requires a Functional Cyclic AMP Response Element, whereas a Different *cis*-Acting Element Mediates Pituitary-Specific Expression *Mol and Cell Biol* **Nov** (Vol 9, No 11): 5113-5122

Braden TD, Farnworth PG, Burger HG, Conn PM (1990) Regulation of the synthetic rate of gonadotropin-releasing hormone receptors in rat pituitary cell cultures by inhibin. *Endocrinology* **127**: 2387-2392

Braden TM, Conn PM (1991) GnRH and its actions. Adapted from Stevenson Lecture *Con J Physiol Pharmacol* 26-54

Brooks J, Crow WJ, McNeilly JR, McNeilly AS (1992) Relationship between gonadotropin subunit gene expression, gonadotropin-releasing hormone receptor content and pituitary and plasma gonadotrophin concentrations during the rebound release of FSH after treatment of ewes with bovine follicular fluid during the luteal phase of the cycle. *J Mol Endo* **8**:109-118

Brooks J, Taylor PL, Saunders PTK, Eidne KA, Struthers WJ, McNeilly AS (1993) Cloning and sequencing of the sheep pituitary Gonadotropin-Releasing Hormone receptor and changes in expression of its mRNA during the estrous cycle. *Mol and Cell Endocrinology* **94**: R23-R27

Burgus R, Butcher M, Ling N, Monahan M, Rivier J, Fellows R, Amoss M, Blackwell R, Vale W, Guillemin R (1971) Structure moleculaire du facteur hypothalamic (LRF) d'origine ovine controlant la secretion de l'hormone gonadotrope hypophysaire de luteinisation (LH). *C.R. Acad. Sci., Paris* **273**:1611-13

Caraty A, Orgeur P, Ghiery JP (1982) Mise en evidence d'une secretion

pulsatile du LH-RH du sang prote hypophysaire chez la Brebis par une technique originale de prelevements multiples. *CR Acad Sci Paris 295*, Serie **111**:103-6

Caraty A, Locatelli A, Martin GB (1989) Biphasic response in the secretion of gonadotropin-releasing hormone in ovariectomized ewes injected with oestradiol. J Endocrinol **123**: 375-82

Carmel, PW, Araki S, Ferin M (1976) Pituitary stalk portal blood collection on rhesus monkeys: evidence for pulsatile release of gonadotropin-releasing hormone (GnRH). *Endocrinology* **99**:243-48

Chakraborty PK, Adams TE, Tarnavsky GK, Reeves JJ (1974) Serum and pituitary LH concentrations in ewes infused with LH-RH/FSH-RH. *J. Anim. Sci.* **39**:1150-1157

Clarke IJ, Cummins JT, deKretser DM (1983) Pituitary gland function after disconnection from direct hypothalamic influences in the sheep. *Neuroendocrinology* **36**: 376-84

Clarke IJ, Cummins JT (1984) Direct pituitary effects of estrogen and progesterone on gonadotropin secretion in the ovariectomized ewe. *Neuroendocrinology* **39**: 267-74

Clarke IJ, Cummins JT (1985) Increased gonadotropin-releasing hormone pulse frequency associated with estrogen-induced luteinizing hormone surges in ovariectomized ewes. *Endocrinology* **116**: 2376-2383

Clarke IJ, Thomas GB, Yao B, Cummins JT (1987) GnRH secretion throughout the ovine estrous cycle. *Neuroendocrinology* **46**:82-88

Clarke IJ, Cummins JT, Crowder ME, Nett TM (1987a) Pituitary receptors for gonadotropin-releasing hormone in relation to changes in pituitary and plasma luteinizing hormone in ovariectomized-hypothalamo pituitary disconnected ewes. I. Effect of changing frequency of gonadotropin-releasing hormone pulses. *Biol Reprod* **37(4):** 749-754

Clarke IJ, Cummins JT, Crowder ME, Nett TM (1988) Pituitary receptors for gonadotropin-releasing hormone in relation to changes in pituitary and plasma luteinizing hormone in ovariectomized-hypothalamo pituitary disconnected Ewes II. A Marked Rise in Receptor Number during the Acute Feedback Effects of Estradiol. *Biol Reprod* **39:** 349-54

Clarke IJ, Cummins JT, Crowder ME, Nett TM (1989) Long-term negative

feedback effects of oestrogen and progesterone on the pituitary gland of the long-term ovariectomized ewe. *J Endocrinol* **120(2):** 207-214

Clarke IJ (1988) Gonadotrophin releasing hormone secretion in anoestrous ewes and the induction of GnRH surges by oestrogen. **J. Endocrinol 117**: 355-60

Clayton RN, Catt KJ (1981) Gonadotropin-Releasing Hormone Receptors: Characterization, Physiological Regulation, and Relationship to Reproductive Function *Endocrine Reviews* Vol 2, No 2: 186-209

Clayton RN, Channabasavaiah K, Sterwart JM, Catt KJ (1982) Hypothalamic regulation of pituitary gonadotropin-releasing hormone receptors: effects of hypothalamic lesions and a gonadotropin-releasing hormone antagonist. *Endocrinology* **110**: 1108-15

Colin IM, Bauer-Dantoin AC, Sundaresan S, Kopp P, Jameson JL (1996) Sexually Dimorphic Transcriptional Responses to Gonadotropin-Releasing Hormone Require Chronic *in vivo* Exposure to Estradiol *Endocrinology* **137**:2300-2307

Conn PM, Crowley WF Jr (1994) Gonadotropin-Releasing Hormone and its Analogs *Annu. Rev. Med.* **45**: 391-405

Conti M, Harwood JP, Dafau ML, Catt KJ (1977) Effect of gonadotropin-induced receptor regulation on biological responses of isolated rat lueal cells. *J Biol Chem* **252**:8869

Coppings RJ, Malaven PV (1976) Biphasic effect of estradiol on mechanisms regulating luteinizing hormone release in ovariectomized ewes. *Neuroendocrinology* **21**: 146-56

Counis R, Maithe C, Jutisz M (1983) Estradiol regulates mRNAs encoding precursors to rat lutropin (LH) and follitropin (FSH) subunits. *Biochem Biophys Res Comm* **114**: 65-72

Crowder ME, Nett TM (1984) Pituitary content of gonadotropoins and receptors for gonadotropin-releasing hormone(GnRH) and hypothalamic content of GnRH during the periovulatory period of the ewe. *Endocrinology* **114(1)**:234-239

Crowder ME, Herring RD, Nett TM (1986) Rapid recovery of gonadotroph function after down-regulation of receptors for GnRH in ewes *J. Reproductive Fertility* **78**:577-585

Culler MD, Megro-Vilar N (1986) Evidence that pulsatile follicle-stimulating hormone and follicle stimulating hormone secretion is independent of endogenous luteinizing hormone-releasing hormone. *Endocrinology* **118**: 609-12

Dalkin AC, Haisenleder DJ, Ortolano GA, Ellis TR, Marshall JC (1989) The frequency of gonadotropin-releasing hormone stimulation differentially regulates gonadotropin subunit messenger ribonucleic acid expression *Endocrinology* **125**:917-924

DiGregorio GB, Kile JP, Herring RH, Nett TM (1991) More rapid restoration of pituitary content of follicle-stimulating hormone than of luteinizing hormone after depletion by oestradiol-17 beta in ewes. *J Repro Fertil* **93(2):** 347-354

DiGregorio GB, Nett TM (1995) Estradiol and progesterone influence the synthesis of gonadotropins in the absence of gonadotropin-releasing hormone in the ewe. *Biology of Reproduction* **53(1):** 166-172

Duello TM, Nett TM (1980) Uptake, localization, and retention of gonadotropin-releasing hormone and gonadotropin-releasing hormone analogs in rat gonadotrophs. *Mol Cell Endocrinol* **19(1):** 101-112

Esbenshade KL, Vogel MJ, Traywick GB (1986) Clearance rate of luteinizing hormone and follicle stimulating hormone from peripheral circulation in the pig. *J Anim Sci* **62**: 1649-1653

Evans RM (1988) *Science* **240**: 889-95

Evans NP, Dahl GE, Mauger D, Karsch FJ (1995) Estradiol induces both Qualitative and Quantitative Changes in the Pattern of Gonadotropin-Releasing Hormone Secretion during the Presurge Period in the Ewe. *Endocrinology* **136**: 1603-9

Findlay JK, Clarke IJ, Robertson DM (1990) Inhibin concentrations in ovarian and jugular venous plasma and the relationship of inhibin with follicle-stimulating hormone and luteinizing hormone during the ovine estrous cycle. *Endocrinology* **126**: 528-35

Fukuda M, Miyamoto K, Hasegawa Y, et al., (1986) Isolation of bovine follicular fluid inhibin of about 32 kDa. *Mol Cell Endocrinol* **44**: 55-60

Gasc J, Baulieu E (1988) Regulation by estradiol of the progesterone receptor

in the hypothalamus and pituitary: an immunohistochemical study in the chicken *Endocrinology* **122**: 1357-65

Glass JD, Amann RP, Nett TM (1984) Effects of season and sex on the distribution of cytosolic estrogen receptors within the brain and the anterior pituitary gland of sheep. *Biol Reprod* **30(4):** 894-902

Goodman RL (1988) Neuroendocrine control of the ovine estrous cycle In: Knobil E, Neill JD, eds. *The physiology of reproduction.* NY: Rave Press, 1988: 1929-69

Green JD, Harris GW (1947) The neurovascular link between the neurohypophysis and adenohypophysis. *J. Endocrinol* **5**:136-146

Gregg DW, Schwall RH, Nett TM (1991) Regulation of gonadotropin secretion and number of gonadotropin-releasing hormone receptors by inhibin, activin-A, and estradiol. *Biology of Reproduction* **44(4):** 725-732

Gregg DW, Allen MC, Nett TM (1990) Estradiol-induced increase in number of gonadotropin-releasing hormone receptors in cultured ovine pituitary cells. *Biology of Reproduction* **43(6):** 1032-1036

Gregg DW, Nett TM (1989) Direct effects of estradiol-17 beta on the number of gonadotropin-releasing hormone receptors in the ovine pituitary. *Biol Reprod* **40(2):** 288-293

Hall SH, Miller WL (1986) Regulation of ovine pituitary glycoprotein hormone α-subunit mRNA by 17β-estradiol in cell culture. *Biol Reprod* **34**: 533-42

Hamernik DL, Clay CM, Turzillo AM, VanKirk EA, Moss GE (1995) Estradiol Increases Amounts of Messenger Ribonucleic Acid for Gonadotropin-Releasing Hormone Receptors in Sheep *Biol of Reprod* **53**: 179-185

Hamernik DL, Kim KE, Maurer RA, Nett TM (1987) Progesterone does not affect the amount of mRNA for gonadotropins in the anterior pituitary gland of ovariectomized ewes. *Biol Reprod* **37(5):** 1225-1232

Hamernik DL, Crowder ME, Nilson JH, Nett TM (1986) Measurement of Messenger Ribonucleic Acid for Luteinizing Hormone β-Subunit, α-Subunit, Growth Hormone, and Prolactin after Hypothalamic Pituitary Disconnection on Ovariectomized Ewes *Endocrinology* **119**:2704-2710

Hamernik DL, Nett TM (1988) Measurement of the amount of mRNA for

gonadotropins during an estradiol-induced preovulatory-like surge of LH and FSH in ovariectomized ewes. *Domes Anim Endocrinol* **5(2):** 129-139

Hamernik DL, Nett TM (1988a) Gonadotropin-releasing hormone increases the amount of messenger ribonucleic acid for gonadotropins in ovariectomized ewes after hypothalamic-pituitary disconnection. *Endocrinology* **122**:959-966

Hamernik DL, Keri RA, Clay CM, Clay JN, Sherman GB, Sawyer RH, Nett TM, Nilson JH (1992) Gonadotrope- and Thyrotrope- Specific Expression of the Human and Bovine Glycoprotein Hormone and α-Subunit Genes Is Regulated by Distinct *Cis*-Acting Elements *Mol Endo* **6**: 1745-1755

Hauger RI, Karsch FJ, Foster DL (1977) A new concept for control of the estrous cycle of the ewe based on the temporal relationships between luteinizing hormone, estradiol and progesterone in peripheral serum and evidence that progesterone inhibits tonic LH secretion. *Endocrinology* **101**:807-817

Henderson KM, Ellen RI, Savage LC, McNatty KP (1989) Studies of the effectiveness of gonadotropin-releasing hormone, steroids and follicular fluid in modulating ovine gonadotropin output *in vivo* and *in vitro*. *J Reprod Fertil* **86**: 105-117

Herring RD, Hamernik DL, Kile JP, Sousa ME, Nett TM (1991) Chronic administration of estradiol produces a triphasic effect on serum concentrations of gonadotropins and messenger ribonucleic acid for gonadotropin subunits, but not on pituitary content of gonadotropins, in ovariectomized ewes. *Biology of Reproduction* **45(1):** 151-156

Herman and Adams (1990) Gonadotropin Secretion in Ovariectomized Ewes: Effect of Passive Immunization against Gonadotropin-Releasing Hormone (GnRH) and Infusion of a GnRH Agonist and Estradiol. *Biol of Reprod* **42**: 273-80

Huang ES, Miller WL (1980) Effects of Estradiol-17β on basal and luteinizing hormone releasing hormone-induced secretion of luteinizing hormone and follicle stimulating hormone by ovine pituitary cell culture. *Biol Reprod* **23**: 124-134

Ibrahim SN, Moussa SM, Childs GV (1986) Morphometric Studies of Rat Anterior Pituitary Cells after Gonadectomy: Correlation of Changes in Gonadotropes with the Serum Levels of Gonadotropins *Endocrinology* **119**: 629-637

Jameson JL, Powers AC, Gallagher GD, Habener JF (1989) Enhancer and Promoter Element Interactions Dictate Cyclic Adenosine Monophosphate Mediated and Cell-Specific Expression of the Glycoprotein Hormone α-Gene. *Molecular Endocrinology* **3**: 763-772

Jensen EV, Jacobson HI (1962) *Recent Prog. Horm. Res* **18**: 387-95

Juengel JL, Guy MK, Tandeski TR, McGuire WJ, Niswender GD (1994) *Biol Reprod* **51**: 380-384

Kaiser UB, Jakubowiak A, Steinberger A, Chin WW (1993) Regulation of rat pituitary gonadotropin-releasing hormone receptor mRNA levels in vivo and in vitro. *Endocrinology*. **133**: 931-34

Karsch FJ, Legan SJ, Ryan KD, Foster DL (1980) Importance of estradiol and progesterone in regulating LH secretion and estrous behavior during the sheep estrous cycle. *Biol Reprod* **23**: 404-13

Karsch FJ, Cummins JT, Thomas GB, Clarke IJ (1987) Steroid Feedback Inhibition of Pulsatile Secretion of Gonadotropin-Releasing Hormone in the Ewe. *Biol Reprod* **36**: 1207-18

Keri RA, Andersen B, Kennedy GC, Hamernik DL, Clay CM, Brace AD, Nett TM, Notides AC, Nilson JH (1991) Estradiol Inhibits Trascription of the Human Glycoprotein Hormone α-Subunit Gene Despite the Absence of a High Affinity Binding Site for Estrogen Receptor *Mol Endo* **5**: 725-33

Khalid M, Haresign W, Hunter MG (1987) Pulsatile GnRH administration stimulates the number of pituitary GnRH receptors in seasonally anoestrous ewes. *J. Reprod Fertil* **79**: 223-30

Kile JP, Nett TM (1994) Differential secretion of follicle-stimulating hormone and luteinizing hormone from ovine pituitary cells following activation of protein kinase A, protein kinase C, or increased intracellular calcium. *Biology of Reproduction* **50(1):** 49-54

Kile JP, Alexander BM, Moss GE, Hallford DM, Nett TM (1991) Gonadotropin-releasing hormone overrides the negative effect of reduced dietary energy on gonadotropin synthesis and secretion in ewes. *Endocrinology* **128(2):**843-849

Kirk SE, Dalkin AC, Yasin M, Haisenleder DJ, Marshall JC (1994)

Gonadotropin-Releasing Hormone Pulse Frequency Regulates Expression of Pituitary Follistatin Messenger RNA: A Mechanism for Differential Gonadotrope Function. *Endocrinology* **135**:876-880

Knobil E (1980) Neuroendocrine control of the menstrual cycle. *Rec Prog Horm Res* **36**:53-88

Landefeld TD, Maurer RA, Kepa J (1985) Luteinizing hormone β-subunit mRNA amounts increase during the preovulatory surge of luteinizing hormone in the ewe: the highest levels are observed at the completion of the peak. *DNA* **4**: 249-254

Landefeld TD, Kepa J, Karsch F (1984) Estradiol feedback effects on the α subunit mRNA in the sheep pituitary gland: correlation with serum and pituitary luteinizing hormone concentrations. *Proc Natl Acad Sci USA* **81**:1322-26

Landefeld TD, Kepa J (1984) Regulation of LHβ-subunit mRNA in the sheep pituitary gland during different feedback states of estradiol. *Biochem Biophys Res Comm* **122**: 1307-13

Laws SC, Beggs MJ, Webster JC, Miller WL (1990a) Inhibin increases and progesterone decreases receptors for gonadotropin-releasing hormone in ovine pituitary culture. *Endocrinology* **127**: 373-80

Laws SC, Webster JC, Miller WL (1990b) Estradiol Alters the Effectiveness of Gonadotropin-Releasing Hormone (GnRH) in Ovine Pituitary Cultures: GnRH Receptors Versus Responsiveness to GnRH *Endocrinology* **127**:381-386

Leather AT, Studd JW, Watson NR, Holland EF (1993) The prevention of bone loss in young women treated with GnRH analogues with "add-back" estrogen therapy. *Obstet. Gynecol.* **81**:104-107

Leung K, Kim KE, Maurer RA, Landefeld TD (1988) Divergent changes in the concentrations of gonadotropoin β-subunit messenger ribonucleic acid during the estrous cycle of sheep. *Mol Endocrinol* **2**: 272-276

L'Hermite M, Niswender GD, Reichert LE Jr, Midgley AR Jr (1972) Serum Follicle-Stimulating Hormone in Sheep as Measured by Radioimmunoassay *Biology of Reproduction* **6**:325-332

Loumaye E, Catt KJ (1983) Agonist-induced regulation of pituitary receptors for gonadotropin-releasing hormone. *J Biol Chem* **258**:12002-12009

Marian J and Conn MP (1983) Subcellular Localization of the Receptor for Gonadotropin-releasing Hormone in Pituitary and Ovarian Tissue. *Endocrinology* **112**: 104

Marshall JC, Dalkin AC, Haisenleder DJ, Paul SJ, Ortolano GA, Kelch RP (1991)Gonadotropin-releasing hormone pulses: Regulators of gonadotropin synthesis and ovulatory cycles. *Recent Prog Horm Res* **47**: 155:189

Mason AJ, Hayflick JS, Zoeller RT, Young WS, Phillips HS, Nikolics K, Seeburg PH (1986a) A deletion truncating the gonadotropin-releasing hormone gene is responsible for hypogonadism in the hpg mouse. *Science* **234**:1366-1371

Mason AJ, Pitts SL, Nikolics K, Szonyi E, Wilcox JN, Seeburg PH, Stewart TA (1986b) The hypogonadal mouse: reproductive functions restored by gene therapy. *Science* **234**:1372-1378

Mason DR, Arora DD, Mertz LM, Catt KJ (1994) Homologous Down-Regulation of Gonadotropin-Releasing Hormone Receptor Sites and Messenger Ribonucleic Acid Transcripts in αT3-1 Cells *Endocrinology* **135**: 1165-1170

McNeilly AS, Swanston JA, Crwo W, et al. (1989) Changes in the concentrations of inhibin throughout the normal oestrous cycle and after the infusion of FSH. *J Endocrinol* **120**: 295-305

Menon M, Peegel H, Katta V (1985) Estradiol potentiation of gonadotropin-releasing hormone responsiveness in the anterior pituitary is mediated by an increase in gonadotropin-releasing hormone receptors. *Am. J. Obstet. Gynecol.* **151**: 534-540

Mercer JE, Clements JA, Funder JW, Clarke IJ (1987) Rapid and specific lowering of pituitary FSHβ mRNA levels by inhibin. *Mol and Cell Endocrinol* **53**: 251-54

Mercer JE, Clements JA, Funder JW, Clarke IJ (1988) Luteinizing hormone-beta mRNA levels are regulated primarily by gonadotropin-releasing hormone and not by negative estrogen feedback on the pituitary. *Neuroendocrinology* **47**:563-566

Miller WL, Huang ES (1985) Secretion of Ovine Luteinizing Hormone *in Vitro*:

Differential Positive Control by 17β-Estradiol and a Preparation of Procine Ovarian Inhibin *Endocrinology* **117**: 907-11

Miller WL, Wu J (1981) Estrogen regulation of follicle-stimulating hormone production in vitro: species variation. *Endocrinology* **108**: 673-679

Miller WL, Knight MMK, Grimek HJ, Gorski J (1977) Estrogen regulation of follicle stimulating hormone in cell cultures of sheep pituitaries. *Endocrinology* **100**: 1306-1316

Miller WL, Laws SC, Wu JC, Webster JC, Beggs, MJ, Phillips C, Lin L, Guzman K (1990) Regulation of LHRH action and FSH Synthesis by Estradiol, Progesterone, and Inhibin. Chin WW and Borne I, eds. *Glycoprotein Hormones* Serona Symposia, USA 209-225

Miller WL, Wu JC, Sealfon SC (1993) Estradiol, progesterone inhibin and GnRH regulated GnRH receptor mRNA(s) in ovine pituitary cultures. *Proc. 75th Ann. Meeting of the Endocrine Society.* **P. 61.**

Miyamoto K, Hasegawa Y, Fukuda M, et al. (1985) Isolation of porcine follicular fluid inhibin of 32K daltons. *Biochem Biophyus Res Comm* **129**: 396-403

Moenter SM, Caraty A, Locatelli A, Karsch FJ (1991) Pattern of gonadotropin-releasing hormone (GnRH) secretion leading up to ovulation in the ewe: existence of a preovulatory GnRH surge. *Endocrinology* **129**: 1175-82

Moenter SM, Caraty A, Karsch FJ (1990) The estradiol-induced surge of gonadotropin-releasing hormone in the ewe. *Endocrinology* **127**: 1375-84

Moss GE, Crowder ME, Nett TM (1981) GnRH-Receptor Interaction. VI. Effect of Progesterone and Estradiol on Hypophyseal Receptors for GnRH, and Serum and Hypophyseal Concentrations of Gonadotropins in Ovariectomized Ewes. *Biol of Reprod* **25**: 938-44

Moss GE, Nett TM (1980) GnRH interaction with anterior pituitary. IV. Effect of estradiol-17β on GnRH-mediated release of LH from ovine pituitary cells obtained during the breeding season, anestrous season, and period of transition into or out of the breeding season. Biol Reprod **23**: 398-403

Mumford EL, Squires EL, Jasko DJ, Nett TM (1994) Use of gonadotropin-releasing hormone, estrogen, or a combination to increase releasable pituitary luteinizing hormone in early transitional mares. *J Anim Sci* **72(1)**: 174-177

Naik SI, Young LS, Charlton HM, Clayton RN (1985) Evidence for a pituitary site of gonadal steroid stimulation of GnRH receptors in female mice. *J. Reprod Fertil* **74**: 615-24

Nett TM (1987) Function of the hypothalamic-hypophysial axis during the post-partum period in ewes and cows. *J Reprod Fertil Suppl* **34**: 201-213

Nett TM, Cermak D, Braden T, Manns J, Niswender G (1987) Pituitary receptors for GnRH and estradiol, and pituitary content of gonadotropins in beef cows. I. Changes during the estrous cycle. *Domest Anim Endocrinol* **4(2):** 123-132

Nett TM, Crowder ME, Moss GE, Duello TM (1981) GnRH-Receptor Interaction. V. Down-Regulation of Pituitary Receptors for GnRH in Ovariectomized Ewes by Infusion of Homologous Hormone. *Biology of Reproduction* **24**:1145-1155

Nett TM, Akbar AM, Niswender GD (1974) Serum levels of luteinizing hormone and gonadotropin-releasing hormone in cycling, castrated and anestrous ewes. *Endocrinology* **94**: 713-718

Nett TM (1990) Regulation of Genes Controlling Gonadotropin Secretion *J. Animal Science* **68**(Suppl. 2): 3-17

Nett TM, Crowder ME, Wise ME (1984) Role of Estradiol in Inducing an Ovulatory-like surge of Luteinizing Hormone in sheep. *Biol Reprod* **30(5):** 1208-1215

Nett TM, Flores JA, Carnevali F, Kile JP (1990) Evidence for a direct negative effect of estradiol at the level of the pituitary gland in sheep. *Biol Reprod* **43(4):** 554-558

Nilson JH, Nejedlik MT, Virgin JB, Crowder ME, Nett TM (1983) Expression of alpha subunit and luteinizing hormone beta genes in the ovine anterior pituitary. Estradiol suppresses accumulation of mRNAs for both alpha subunit and luteinizing hormone beta. *J Biol Chem* **258(20):** 12087-12090

Niswender GD, Reichert LE Jr, Midgley AR Jr, Nalbandov AR (1969) Radioimmunoassay for bovine and ovine luteinizing hormone *Endocrinology* **84** 1166-1173

Olson PN, Mulnix JA, Nett TM (1992) Concentrations of luteinizing hormone

and follicle-stimulating hormone in the serum of sexually intact and neutered dogs. *Am J Vet Res* **53(5):** 762-766

O'Malley BW, McGuire WL, Kohler PO, Koreman SG (1969) *Recent Prog Horm. Res.* **25**: 105-60

O'Malley BW, Tsai SY, Bagchi MK, Weigel NL, Schrader WT, Tsai MJ (1991) *Recent Prog. Horm. Res.* **47**:1-26

Padmanabhan V, Leung K, Convey EM (1982) Ovarian steroids modulate the self-priming effect of luteinizing hormone releasing hormone on bovine pituitary cells in vitro. *Endocrinology*. **110**: 717-721

Papavasiliou SS, Zmeili S, Khoury S, Landefeld TD, Chin WW, Marshall JC (1986) Gonadotropin-releasing hormone differentially regulates expression of the genes for luteinizing hormone alpha and beta subunits in male rats. *Proc Natl Acad Sci USA* **83**:4026-4029

Parrot RF, Davies RV (1979) Serum gonadotroin levels in prepubertally castrated male sheep treated for long periods with proprionated testosterone, dihydrotestosterone, 19-hydroxytestosterone or oestradiol. *J Reprod Fertil* **56**: 543-48

Phillips CL, Lin LW, Wu JC, Guzman K, Milsted A, Miller WL (1988) 17 β-Estradiol and progesterone inhibit transcription of the genes encoding the subunits of ovine follicle-stimulating hormone. *Mol Endocrinol* **2**: 641-49

Pierce JG, Parsons TF (1981) Glycoprotein hormones: structure and function. *Annu Rev Biochem* **50**: 465-495

Reeves JJ, Arimura A, Schally AV (1971) Changes in pituitary responsiveness to luteinizing hormone-releasing hormone (LH-RH) in anestrous ewes pretreated with estradiol benzoate. *Biol Reprod* **4**: 88-92

Rhodin JA (1974) Histology, a text and atlas. Oxford Univ Press, NY, pp. 427-440

Rippel RH, Johnson ES, White WF (1974) Effect of consecutive injections of synthetic gonadotropin-releasing hormone on LH release in anestrous and ovariectomized ewes. *J Animal Sci* **39**:907

Risma KA, Clay CM, Nett TM, Wagner T, Yun J, Nilson JH (1995) Targeted

over expressionof luteinizing hormone in transgenic mice leads to infertility, polycystic ovaries, and ovarian tumors. *Proc Natl Acad Sci USA* **92(5):** 1322-1326

Roberts VJ, Peto CA, Vale W, Sawchenko PE (1992) Inhibin/activin subunits are costored with FSH and LH in secretory granules of the rat anterior pituitary gland. *Neuroendocrinology* **56**: 214-224

Roche JF, Foster DL, Karsch FJ, Cook B, Dziuk PJ (1970). Levels of luteinizing hormone in sera and pituitaries of ewes during the estrous cycle and anestrus. *Endocrinology* **86**: 568-72

Sakukrai H, Adams BM, Adams TE (1992) Pattern of gonadotropin-releasing hormone (GnRH)-like stimuli sufficient to induce follicular growth and ovulation in ewes passively immunized against GnRH *Biol Reprod* **45**: 804-810

Sakurai H, Adams, BM, Adams TE (1996) Gonadotrope responsiveness in orchidectomized sheep: effect of duration of a simulated follicular phase. *Journal of Reproduction and Fertility* **108**:211-217

Sakurai H, Adams BM, Adams TE (1997) Concentration of Gonadotropin-Releasing Hormone Receptor Messenger Ribonucleic Acid in Pituitary Tissue of Orchidectomized Sheep: Effect of Passive Immunization Against Gonadotropin-Releasing Hormone. *J Anim Sci* **75**: 189-194

SAS (1987) SAS User's Guide, Statistics. Statistical Analysis System Institute, Inc., Cary NC

Savoy-Moore RT, Schwartz NB,Duncan JA, Marshall JC (1980) Pituitary gonadotropin-releasing hormone receptors during the rat estrous cycle. *Science* **209**: 942-44

Schally AV, Arisura A, Baba Y, Nair RMG, Matsuo H, Redding TW, Debeljuk L (1971) Isolation and Properties of the FSH and LH-Releasing Hormone *Biochemical and Biophysical Research Communications* **43**:(No. 2):393-399

Schally AV, Kastin AJ, Arimura A (1972) FSH-releasing hormone and LH-releasing hormone. *Vitamins and Hormones* **30**:83-164

Schanhacher BD, Ford JJ (1977) Gonadotropin secretion in cryptochid and castrate rams and the acute effects of exogenous steroid treatment. *Endocrinology* **100**: 387-93

Schoenemann HM, Humphrey WD, Crowder ME, Nett TM, Reeves JJ (1985) Pituitary luteinizing hormone-releasing receptors in ovariectomezed cows after challenge with ovarian steroids. *Biol Reprod* **32(3):** 574-583

Schuiling GA, De Koning J, Zurcher AF, Gnodde HP, Van Rees GP (1976) Induction of LH Surges by Continuous Infusion of LH-RH *Neuroendocrinology* **20**:151-156

Sealfon SC, Laws SC, Wu JC, Gillo B, Miller WL (1990) Hormonal regulation of gonadotropin-releasing hormone receptors and messenger RNA activity in ovine pituitary culture. *Mol Endocrinol.* **4**: 1980-7

Simone D.A., Mahesh V.B (1993) An autoregulatory process for androgen production in rat thecal-interstitial cells. *Biol. Reprod.* **48**:46-56

Sternberger LA, Petrali JP (1975) quantitative immunocytochemistry of pituitary receptors for luteinizing hormone-releasing hormone. *Cell Tissue Res* **162**: 141-76

Tamanini C, Crowder ME, Nett TM (1986) Effects of oestradiol and progesterone on pulsatile secretion of luteinizing hormone inovariectomized ewes. *Acta Endocrinol (Copenh)* **111(2):** 172-178

Tamanini C, Crowder ME, Nett TM (1988) Progesterone does not inhibit the increase in pituitary content of luteinizing hormone after removal of estradiol in the ewe. *Acta Endocrinol (Copenh)* **118(2):** 193-198

Tsai M, O'Malley BW (1994) Molecular Mechanisms of Action of Steroid/Thyroid Receptor Superfamily Members *Ann. Rev. Biochem* **63**: 451-86

Turzillo AM, Nett TM (1995) Effects of estradiol on concentrations of gonadotropin-releasing hormone receptor messenger ribonucleic acid following removal of progesterone. *Endocrine* **3**: 765-68

Turzillo AM, Di Gregorio GB, Nett TM (1995) Messenger Ribonucleic Acid for Gonadotropin-Releasing Hormone Receptor and Numbers of Gonadotropin-Releasing Hormone Receptors in Ovariectomized Ewes after Hypothalamic-Pituitary Disconnection and Treatment with Estradiol *J Animal Science* **73**:XXX

Turzillo AM, Juengel JL, Nett TM (1995a) Pulsatile gonadotropin-releasing

hormone (GnRH) increases concentrations of GnRH receptor messenger ribonucleic acid and numbers of GnRH receptors during luteolysis in the ewe. *Biology of Reproduction* **53**: 418-423

Turzillo AM, Campion CE, Clay CM, Nett TM (1994) Regulation of gonadotropin-releasing hormone (GnRH) receptor messenger ribonucleic acid and GnRH receptors during the early preovulatory peroid in the ewe. *Endocrinology* **135**: 1353-58

Vaitukaitis JL (1979) Human chorionic gonadotropin—a hormone secreted for many reasons. *N Engl J Med* **301**: 324-326

Vizcarra JA, Wettemann RP, Braden TD, Turzillo AM, Nett TM (1997) Effect of gonadotropin-releasing hormone (GnRH) pulse frequency on serum and pituitary concentrations of luteinizing hormone and follicle-stimulating hormone, GnRH receptors, and messenger ribonucleic acid for gonadotropin subunits in cows *Endocrinology* **138(2):** 594-601

Wang QF, Farnworth PG, Findlay JK, Burger HG (1988) Effect of purified 31K bovine inhibin on the specific binding of gonadotropin-releasing hormone to rat anterior pituitary cells in culture. *Endocrinology* **123**:2161-6

Westman A, Jacobsohn D (1940) Esperimentille untersuchung uber hypophysentransplantate bei der ratte. *Acta. Pathol. Microbiol. Scan.* **17**:328-347

Wheaton JE (1979) Regional brain content of luteinizing hormone-releasing hormone in sheep during the estrous cycle, seasonal anestrous and after ovariectomy. *Endocrinology* **104**:839-844

Windle JJ, Weiner RI, Mellon PL (1990) Cell Lines of the Pituitary Gonadotrope Lineage Derived by Target Oncogenesis in Transgenic Mice. *Molecular Endocrinology* **4**: 597-603

Wise ME, Nieman D, Stewart J, Nett TM (1984) Effect of number of receptors for gonadotropin-releasing hormone on the release of lutenizing hormone. *Biol Reprod* **31(5):** 1007-1013

Wise ME, Nilson JH, Nejedlik MT, Nett TM (1985) Measurement of messenger RNA for luteinizing hormone beta-subunit and alpha subunit during gestation and the postpartum period in ewes. *Biol Reprod* **33(4):**1009-1015

Wise ME, Sawyer HR Jr, Nett TM (1986a) Functional changes in luteinizing

hormone-secreting cells from pre- and postpartum ewes. *Am J Physiol* **250(3 Pt 1):** E282-E287

Wise ME, Glass JD, Nett TM (1986) Changes in the concentration of hypothalamic and hypophyseal receptors for estradiol in pregnant and postpartum ewes. *J Anim Sci* **62(4):** 1021-1028

Wu JC, Sealfon SC, Miller WL (1994) Gonadal hormones and gonadotropin releasing hormone (GnRH) alter messenger ribonucleic acid levels for GnRH receptors in sheep. *Endocrinology* 134: 1846-50

Wun WA, Thorneycroft LH (1987) Estradiol positive feedback on the rat anterior pituitary gland in vitro. *Mol Cell Endoc* **54**: 165-69

Yamato KR (1985) *Annu. Rev. Genet.* **19**:209-52

Yen SSC, Vanaenberg G, Siler TM (1974) Modulation of pituitary responsiveness to LRF by estrogen. *J Clin Endocrinol Metab* **39**: 170-177